PB-218 474

CHLOROMELAMINE FORMULA 96P - BACTERICIDAL CHEMICAL AND PHYSICAL PROPERTIES

Milton J. Foter

Enviromental Health Center
Cincinnati, Ohio

July 1952

DISTRIBUTED BY:

NTIS
National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

8-145 516

LIBRARY
ENVIRONMENTAL HEALTH CENTER
CINCINNATI, OHIO
PB 218 474

FINAL REPORT

on

CHLOROMELAMINE FORMULA 96P - BACTERICIDAL
CHEMICAL AND PHYSICAL PROPERTIES

July - 1952

of the

Research and Development Branch
Environmental Health Center
U. S. Public Health Service
Cincinnati, Ohio

for

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

USAF - AIR MATERIAL COMMAND

Wright-Patterson Air Base

Under Contract No. AF-33(038)22425

110

Submitted by:

Milton J. Foter
Milton J. Foter
Associate Chief

George R. Weber
George R. Weber
Associate Chief
Sanitizing Agents Unit
Bacteriology Section

Lloyd R. Setter
Lloyd R. Setter
Assistant Chief
Physics and Chemistry Section

Forwarded:

Paul W Kabler
Paul W. Kabler
Chief, Bacteriology Section

Leslie A. Chambers
Leslie A. Chambers
Director of Research

V.G. MacKenzie
V.G. MacKenzie
Officer in Charge
Environmental Health Center

CONTENTS

I SUMMARY

II BACTERICIDAL PROPERTIES OF CHLOROMELAMINE FORMULA 96P

PART A. General

PART B. Basic Studies

1. Methods and Procedures
2. Effect of Concentration
3. Effect of Reaction (pH)
4. Effect of Temperature
5. Effect of Organic Matter
6. Basic Studies - Conclusions

PART C. Stability Studies

PART D. Disinfection of Mess Gear

PART E. Disinfection of Raw Vegetables

III CHEMICAL AND PHYSICAL PROPERTIES OF CHLOROMELAMINE FORMULATIONS

PART A. Introduction

PART B. Basic Studies

1. Methods and Procedures
2. Composition of Chloromelamine Formulations
3. Comparative Stability of Solutions of Chloromelamine and Hypochlorous Acid
4. Shelf Stability Studies
5. Summary and Discussion

I SUMMARY

This study was initiated to determine the suitability and germicidal efficiency of Chloromelamine Formula 96P for use by the Air Force for disinfection of mess gear, fresh vegetables, and for possible use as a general disinfectant.

Results of basic studies indicated that, in distilled water, doubling the available chlorine concentration from 12.5 ppm up to 200 ppm reduced the killing times approximately one half. In the presence of synthetic buffered hard water, containing 2000 ppm peptone, increasing the concentration from 50 ppm to 100 ppm available chlorine reduced the killing time to about one third; further increases of available chlorine to 200 ppm reduced the killing time to 1/12 its value at 100 ppm.

The germicidal efficiency of chloromelamine (two formulations) was reduced as the pH increased. In preparations of greater than pH 7, chloromelamine showed marked losses of germicidal activity as the solution aged. These losses progressively increased up to pH 10. It was therefore necessary to buffer chloromelamine formulations at low pH values to maintain germicidal efficiency.

In a solution containing 200 ppm available chlorine, 10°C. drop in temperature approximately doubled the time required to kill 99.9999% of the test organism. Chloromelamine Formula 96P failed to effect a 99.999% reduction of *Escherichia coli* within 20 seconds at either 5°C. or 50°C., as prescribed in a test developed by Morse and Feder, and recommended by the National Research Council, Subcommittee on Food Supply, for germicides considered for military use.

In the presence of 1000 ppm food soil (based on U.S. Army field ration) and at 25°C., Formula 96P at 200 ppm available chlorine killed 99.9999% of the test organisms in 0.41 minutes (25 seconds). In the absence of organic matter and other conditions unchanged, the killing time for Formula 96P was about the same.

Formula 96P stored in brown glass bottles, plastic bags and cardboard boxes at 160°F. and 90 to 100% humidity deteriorated rapidly. Samples stored at 160°F. at 6% humidity deteriorated gradually; some were unsuitable at about 60 days and all were unusable after 174 days. At room temperature (about 75°F.) and uncontrolled humidity, samples showed slight deterioration after 194 days. Samples stored at 32°F. and - 65°F. showed no deterioration in 175 days.

In the absence of organic matter, there was good correlation between the available chlorine content (starch - iodide method) and the germicidal activity of undeteriorated Formula 96P. In deteriorating samples correlation of the two tests became progressively poorer.

Results of applied studies in the disinfection of mess gear showed that Sulframin AB in the wash water and Formula 96P in the rinses were slightly less efficient than G.I. soap and hypochlorite under similar conditions of test.

Formula 96P at a concentration of 200 ppm available chlorine at either 5°C. or 25°C. did not remove all viable coliform organisms from contaminated vegetables. However, exposure of contaminated tomatoes and carrots to 200 ppm available chlorine reduced the coliform counts to levels lower than those found on control market samples. Numbers of contaminating organisms remaining on celery and lettuce after disinfection were much greater than those on control market samples.

SECTION II

Bactericidal Properties
of
Chloromelamine Formula 96P

Part A

Introduction

There has been a need for an improved germicidal rinse compound for use by military forces in the field. A germicidal rinse compound should be one that will serve all ordinary military purposes in the field such as the disinfection of mess kits under emergency conditions and the disinfection of raw fruits and vegetables which may have become contaminated with human pathogens.

At the request of the Office of the Surgeon General of the Army for recommendations on the military requirements for a germicidal rinse compound, the Subcommittee on Food Supply, Committee on Sanitary Engineering and Environment, National Research Council in its report of 10 January, 1952 recommended that the following requirements be adopted:

The germicidal rinse compound shall:

1. Be a non-liquid halogen compound or compounds.
2. Be sufficiently soluble in water at 5°C. with slight initial stirring so that after standing not more than 15 minutes a portion of the solution decanted from near the surface will give the bactericidal efficiency herein required.
3. Show by test a 99.999 per cent reduction of a selected strain of Escherichia coli within 20 seconds contact under the following conditions:
    - a. When mixed with water in prescribed proportions.
    - b. When the solution contains at least 100,000,000 Escherichia coli per ml.
    - c. When the solution contains 0.2 per cent of peptone, 0.054 per cent of bicarbonate, and 0.036 per cent of calcium chloride.
    - d. When tested at 5° and 50°C. from 15 to 120 minutes after mixing.
4. When properly packaged, be stable under the usual conditions of shipment and storage for the maximum interval between procurement and use to be designated.

5. Be so packaged that each unit will contain sufficient compound to maintain an effective bactericidal level of the halogen when dissolved in 25 gallons of water.

6. If partitionally packed, be so sealed that breaking the seal will expose all components for emptying into the rinse water simultaneously.

7. Be non-toxic and non-irritating under conditions of use.

8. Not unduly corrode the sinks or containers in which vegetables may be washed, the cans provided for germicidal rinse solutions, nor the metal in mess kits.

9. Be compatible with soap or the washing compound issued for use in conjunction with the germicidal rinse compound.

10. Contain sufficient surface active agent to give, in use dilution, a surface tension of not more than 40 dynes.

11. Be buffered to maintain an acidity no greater than pH 3.0 when dissolved in distilled water.

12. Be designated for use in both the intermediate and final rinse waters in treating mess kits, and in both a preliminary rinse or wash and in the final rinse water in treating vegetables.

At the request of the Air Material Command a study was initiated to determine the effectiveness of chloromelamine as a general purpose bactericidal agent to be used on land and in the air, over a wide range of conditions. Pursuant to this study, a meeting was held on April 24, 1951 in the office of the Director of Research, Environmental Health Center, in which Environmental Health Center representatives and a representative from the Wallace & Tiernan Co., Inc. (manufacturer of chloromelamine) participated. At that time orders were placed with the Wallace & Tiernan Co., Inc. for Chloromelamine Formula 96P (as per proposed specifications Office of the Army Quartermaster General, dated 2 March, 1951), to be packaged (100 grams each) as follows:

brown glass bottles	144 each
cardboard boxes	144 each
plastic bags	144 each

This material was received for study on or about September 11, 1951.

Formula 96P * (percentage by weight) was as follows:

Chloromelamine	22.0
Surface active material #	11.5 - 12.5
Citric acid, anhydrous	51.0 - 55.0
Monosodium dihydrogen phosphate, anhydrous	10.5 - 15.5

Prior to the manufacture of Formula 96P, a wetting agent mixture with no citric acid was received for study (designated WAM or 55-5). This mixture was used in the preliminary concentration and pH studies until Formula 96P became available. The wetting agent mixture formula with percentages by weight was as follows:

Chloromelamine	56.0
Sulframin AB-85	4.6
Monosodium dihydrogen phosphate, anhydrous	39.4

There are six amino hydrogens on a molecule of chloromelamine, and if all six of these hydrogens were replaced in the chlorination process, the solubility of the resulting product would be too low to be of practical value as a germicidal agent. Chloromelamine is a chlorination product of 1, 3, 5 triamino triazine (melamine) in which three amino hydrogens are replaced by chlorine.

* As described in (Proposed) Military Specification, Disinfectant, Chlorine, Food Service, MIL (QMC), 2 March, 1951.

# Sulframin AB-85, Ultra Chemical Works, Inc., Paterson, N.J.

While chloromelamine was not superior to sodium hypochlorite as a germicide, it was reported to have the advantages of stability on storage and of maintaining active chlorine in the presence of organic matter such as that found in food wastes. Like other halogen compounds, chloromelamine was reported to be more active in acid than in alkaline solution and thus Formula 96P was acid buffered.

Following conferences with representatives of the Surgeon's Office, Air Material Command, the following plan of study was outlined:

I. Basic studies with Formula 96P or the WAM Formula.
  a. Effect of concentration.
  b. Effect of pH.
  c. Effect of temperature.
  d. Effect of organic matter.
II. Applied Screening Studies with Formula 96P.
  a. Efficiency in disinfection of mess kits under normal and adverse conditions (organic matter and low temperature).
  b. Efficiency in the disinfection of contaminated raw vegetables.
III. Stability Studies.
  a. Storage of Formula 96P packaged in (1) brown glass bottles, (2) cardboard boxes and (3) plastic bags at temperature-humidity combinations as follows:

Combination	Temp.	Relative humidity	Location
A	160°F.	90-100%	Wright-Patterson Equipment Lab.
B	160°F.	6%	"
C	Room Temp. (about 75°F.)	Uncontrolled *	Cincinnati (Faller Bldg.)
D	32 - 35°F.	Uncontrolled	Wright-Patterson Equipment Lab.
E	- 65°F.	Uncontrolled	"

Bottled samples of Formula 96P were received directly from Wallace and Tiernan Co., whereas samples in cardboard boxes and plastic bags were from the same Wallace and Tiernan lot of chloromelamine but were packaged by Economics Laboratory, Inc. Samples were taken to Wright-Patterson Equipment Laboratory on September 12, 1951 for storage. After an initial delay in getting the equipment into operation, samples were placed in storage on October 4,1951. Plans were made to withdraw samples periodically for bactericidal and chemical tests.

* Designated as "normal humidity" in Section III.

Part B

Basic Studies

1. Methods of Procedures

In the basic studies, the germicidal activity of chloromelamine was determined by the procedure previously employed by Weber (Public Health Reports, 65, No. 15, April 14, 1950, page 503) which is a modification of that reported by Weber and Black (American Journal of Public Health, 38, No. 10, October 1948, page 1405). In brief, this procedure involved the establishment of a survival-curve, in such a manner that the time required to kill 99.9999% of the bacteria became a measure of the germicidal efficiency. The test culture employed was an agar slant growth of Micrococcus pyogenes var. aureus (Staphylococcus aureus). Incubation was at 35°C. for 24 hours. The selection of this test organism was based on earlier studies of germicidal activity of chlorine compounds in which it was observed that the resistance of the culture was quite satisfactory for giving killing times in measurable ranges.

The number of bacteria per ml. of test solution was established at 100 million and the time required to kill 99.9999% of the bacteria indicated the approximate time that would be required to sanitize a heavily contaminated food utensil such as a field mess kit.

The germicide mixture was prepared by weighing a sample of Formula 96P and diluting it so as to prepare a solution slightly more than double the test concentration required. This solution of approximately double concentration was titrated using the sodium thiosulphate-starch iodide procedure and standardized to exactly double the test concentration required. Following this, the solution was brought to the test temperature by placing it in a constant temperature water bath.

Where specified, the synthetic hard water was prepared by the addition of 10 ml. each of a 3.6% solution of calcium chloride and a 5.4% solution of sodium bicarbonate to sterile distilled water and made up to 1000 ml. (Military Spec. MIL-D-11309 (QMC), 6 August, 1951).

Dilution blanks (9 ml.) were employed for neutralizing 1 ml. aliquots removed from the medication tube after each exposure interval. These blanks consisted of a solution of phosphate buffer, lecithin, a non-ionic surface active agent to inactivate the anionic surface active agent, and a chlorine neutralizing agent (sodium thiosulphate or sodium sulfite). Dilution blanks were prepared and adjusted with acid or alkali, as the case may be, so that when the aliquot from the medication tube was introduced, the solution came immediately to neutrality.

It has been observed that sodium thiosulphate breaks down and thus is not available for chloromelamine neutralization when sterilized in the presence of other materials in the blank. Sodium sulfite will not withstand head sterilization and should be prepared "aseptically" in sterile distilled water just prior to use.

In the study of the effect of pH on the germicidal activity of chloromelamine, it was necessary to prepare special buffers. The procedure employed was essentially that reported by Weber and Levine, (American Journal of Public Health, 34, No. 7, July 1944, page 719). Buffers employed were as follows: pH 11, 10 and 9, carbonate; pH 8, 7 and 6, phosphate; pH 5, acetate; pH 4, phthalate. Since some of the buffer salts were not heat stable it necessitated aseptic preparation of certain solutions and heat sterilizing only the stable materials.

Just prior to each experiment, a plating was made of the culture suspension to determine the initial count. At the zero time on each experiment, when all solutions were at the required temperature, 5 ml. of the double strength germicide was introduced into the medication tube which contained 5 ml. of a double concentration of bacteria. The solution was swirled for immediate mixing and at appropriate time intervals, 1 ml. aliquots were withdrawn and introduced into the 9 ml. neutralization blanks (for neutralizing chloromelamine, acid or alkali and surface active agent). The blanks containing the surviving bacteria were plated in the required dilution. After each individual or series of experiments, a plating was again made of the culture suspencion to ascertain whether the culture had changed in numbers during the experiment or experiments. Generally, three experiments were made for each condition.

Following each experiment, chloromelamine titrations and pH determinations were made for the solution employed in the medication tube and for the dilution blanks. After 48 hours'incubation at 35°C., plate counts were made. The time required to kill 99.9999% of the exposed bacteria was determined by plotting the logarithm of the percentage of surviving bacteria against exposure time.

Control tests were made at each pH level, to establish that the buffer itself was not toxic to the organisms. This was accomplished by exposing the test organism to buffer in the absence of chloromelamine.

The resistance of the test culture was checked periodically by establishing survival curves and killing times, employing a constant alkaline hypochlorite compound as the standard.

2. Effect of Concentration

Tables I and II show the effect of concentration of Formula 96P and Wetting Agent Mixture in distilled water and synthetic water on pH and killing time (99.9999%) of Micrococcus pyrogenes var. aureus at 25°C. The Wetting Agent Mixture was available for study at a date earlier than Formula 96P and was used to show the effect of two variables (concentration and pH) on the killing time. The theoretical concentrations of available chlorine and the actual concentrations are listed as determined by the thiosulphate-starch iodide titration.

Table I and Figures 1-A and 1-B show the effect of concentration of Chloromelamine Formula 96P on the killing time of the test organism at 25°C. using distilled water and the synthetic hard water recommended by the National Research Council. Killing times in distilled water were doubled approximately by halving the available chlorine concentration. In the presence of the synthetic hard water containing peptone, the killing times were all increased and the trend of a unit increase in killing time with a unit reduction in germicide concentration was not as evident. In the presence of 2000 ppm of peptone at 200 ppm available chlorine the killing time was only one minute, despite the face that the test solution contained 10 times as much peptone as available chlorine.

With the Wetting Agent Mixture (Table II), between theoretical concentrations of 400 and 100 ppm available chlorine the killing time again doubled approximately as the concentration was halved. This formulation was poorly buffered and as the concentration was dropped below the theoretical level of 100 ppm available chlorine the pH changed rapidly and exerted pronounced effect upon the killing time. The increase in pH with dilution of the germicide indicates the need for the inclusion of buffers in any formulation.

TABLE I

EFFECT OF CONCENTRATION OF CHLOROMELAMINE FORMULA 96P ON THE KILLING TIME (99.9999%) Of *Micrococcus pyogenes* var. *aureus*: 25°C.

Theoretical Concentration PPM. Av.Cl.	Actual Concentration PPM. Av.Cl.[1]	pH ± 0.1	Killing time in Minutes[1]
	DISTILLED WATER		
200	190	3.0	0.10
100	97	3.0	0.23
50	46*	3.0	0.39
25	25	3.1	0.55
12.5	12.5	3.0	0.93
	SYNTHETIC WATER[2]		
200	200	4.7	1.0
100	94	5.9	12.4
50	43**	6.6	39.
	44**	6.6	> 30
25	20*	7.0	> 60

1. Each value is the mean of three or more experiments unless otherwise noted.

* - Mean of two experiments.

** - One experiment.

2. 360 ppm. $CaCl_2$, 540 ppm $NaHCO_3$, 2000 ppm peptone in distilled water.

TABLE II

EFFECT OF CONCENTRATION OF CHLOROMELAMINE WETTING AGENT MIXTURE[1] IN DISTILLED WATER ON pH AND KILLING TIME (99.9999%) Of *Micrococcus pyogenes* var. *aureus*: 25°C.[2]

Theoretical Concentration PPM Av. Cl.	Actual PPM Available Chlorine	pH	Killing Time in Minutes
400	397	3.8 ±0.1	0.09
200	197	3.9 ±0.1	0.17
100	96	4.3 ±0.3	0.27
50	48	4.5 ±0.3	1.4
25	25	4.8 ±0.1	3
12.5	12	5.6 ±0.2	33
6.25	6	5.7 ±0.2	89
3.13	3	6.1 ±0.1	141

---

[1] Wetting Agent Mixture contained no citric acid.

[2] Each value except pH is the mean of 3 or more experiments.

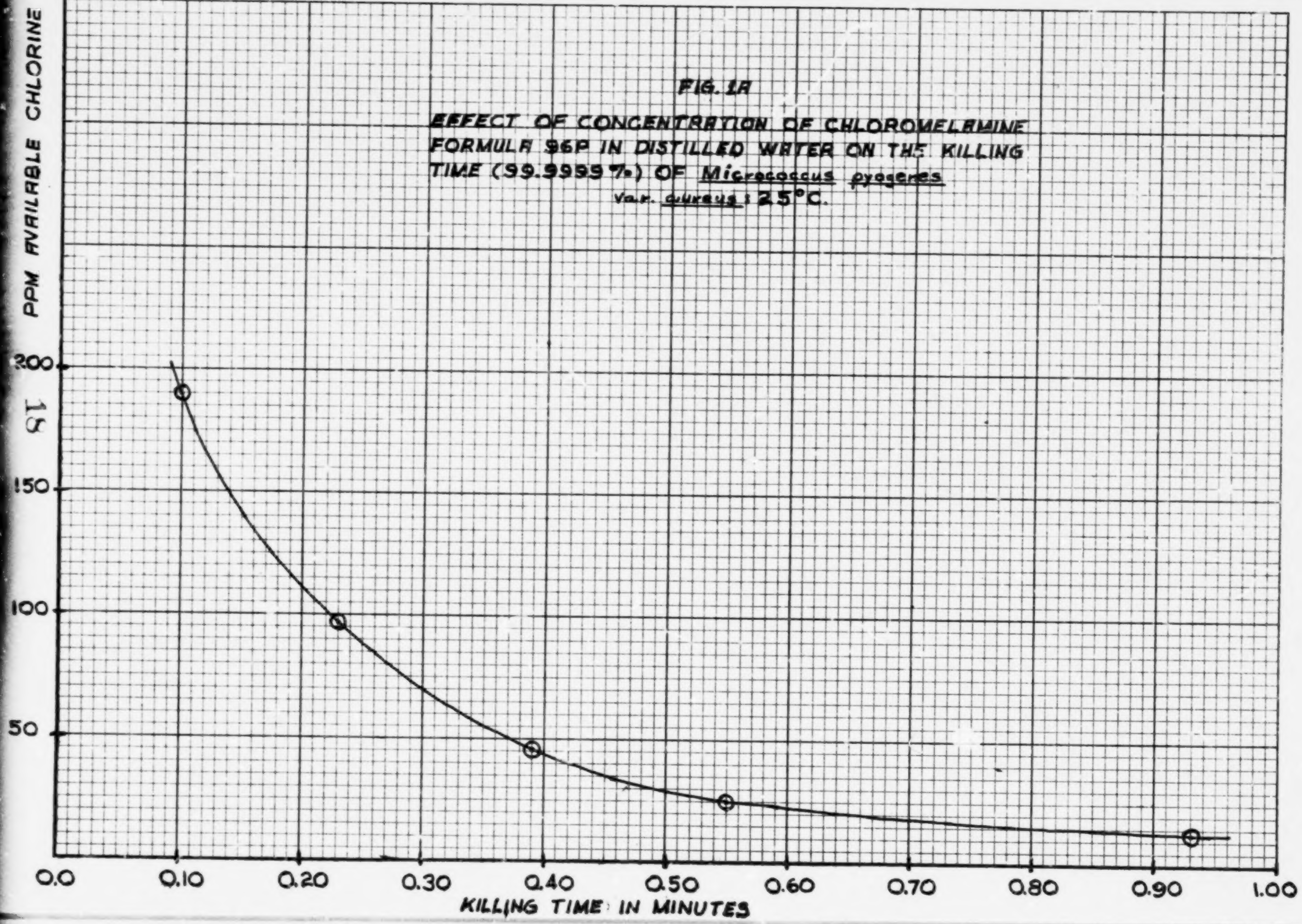
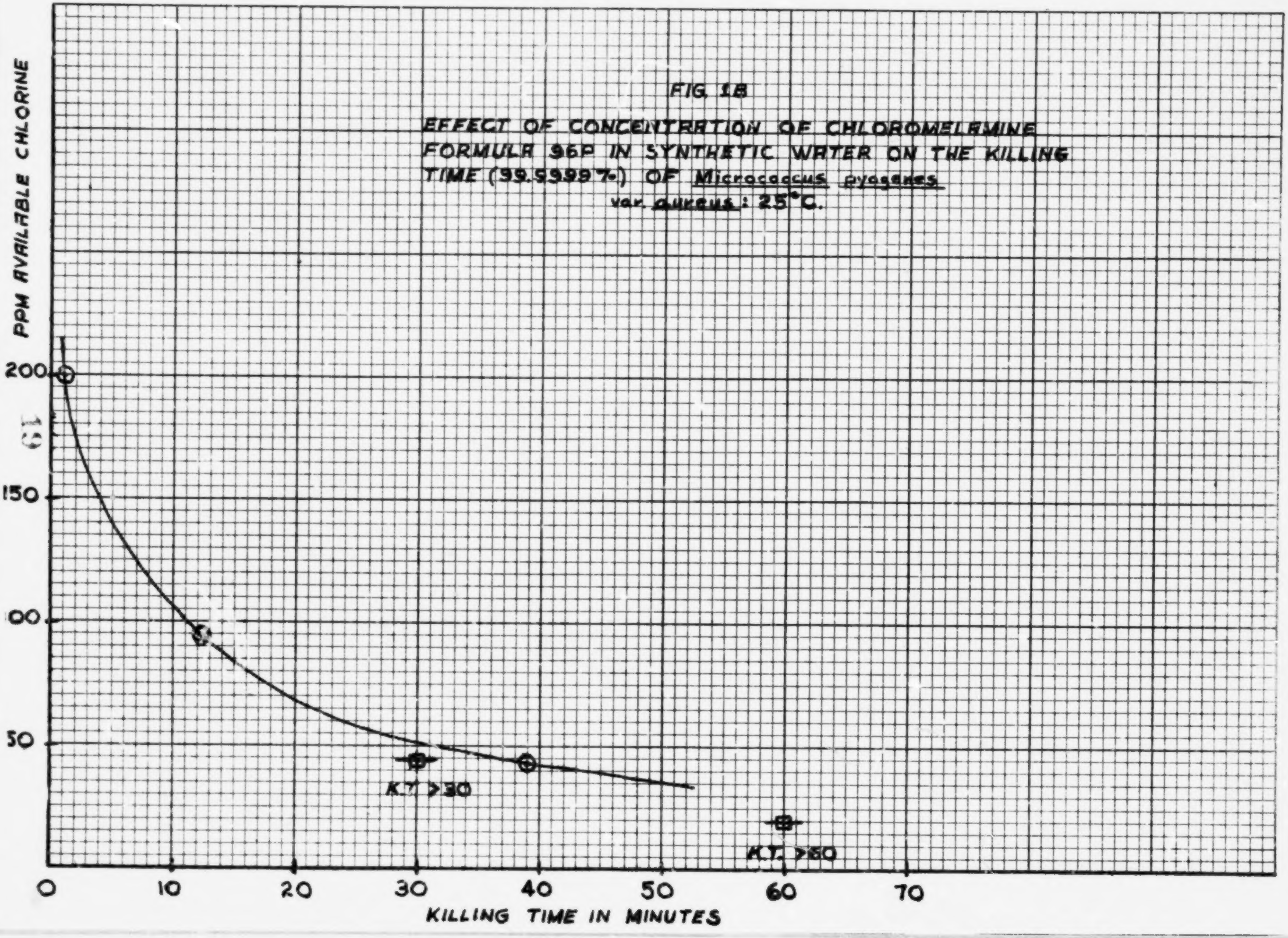


FIG. 1A

EFFECT OF CONCENTRATION OF CHLOROMELAMINE FORMULA 96P IN DISTILLED WATER ON THE KILLING TIME (99.9999%) OF *Micrococcus pyogenes* var. *aureus* : 25°C.

FIG. 18

EFFECT OF CONCENTRATION OF CHLOROMELAMINE FORMULA 96P IN SYNTHETIC WATER ON THE KILLING TIME (99.9999%) OF *Micrococcus pyogenes* var. *aureus*: 25°C.



3. Effect of Reaction (pH)

Table III shows the effect of pH and age of solution (200 ppm available chlorine) of the Wetting Agent Mixture in distilled water on the killing time (99.9999%) of Micrococcus pyogenes var. aureus at 25°C. and at pH values of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0.

The Wetting Agent Mixture containing chloromelamine with no citric acid was secured primarily to study the effect of pH on the germicidal action of chloromelamine. The presence of citric acid in a formulation such as 96P made buffering more difficult. Results shown in Table IV at approximately pH 5 demonstrate that killing times with the WAM formulation were approximately the same as for 96P at comparable pH and concentration levels. When Formula 96P was adjusted with calcium chloride and sodium bicarbonate and when the Wetting Agent Mixture was adjusted with acetate buffer there were no significant differences in killing times as compared with Formula 96P adjusted with phosphate buffer.

In this phase of the study rather great variations in killing time occurred with alkaline solutions of chloromelamine formulations, depending upon the time elapsing between addition of the buffer to adjust pH and the time of running the experiment to determine the killing time. The time a germicide is held at a specific pH may be of great practical importance. A study was included to simulate the time which Formula 96P solutions might be held when sanitizing mess kits. The germicidal activity at 25°C. was determined within 5 minutes, one hour, and two hours after buffering.

The data indicate that changes which take place in chloromelamine solutions buffered to alkaline reactions occur rapidly - within a few minutes, with a loss of germicidal activity. The data recorded in Table III under "Age of Solution" of approximately 5 minutes presumably represent largely unchanged chloromelamine.

TABLE III

EFFECT OF REACTION (pH) AND AGE OF SOLUTION OF CHLOROMELAMINE W.A.M. IN DISTILLED WATER (200 PPM AV.CL.) ON THE KILLING TIME (99.9999%) OF Micrococcus pyogenes var. aureus: 25°C.

pH[1]	Age of Solution in Min.[2]	PPM of Available Chlorine	Killing Time in Min.
4.0	≈5	199	0.24
4.0	60[x]	193	0.15
4.0	120	198	0.52
5.0	≈5	201	0.15
5.0	60[x]	199	0.21
5.0	120[x]	201	0.12
6.0	≈5	194	0.60
6.0	60[x]	198	2.0
6.0	120	199	1.2
6.0	300[x]	195	1.7
7.0	0.25	194	0.26
7.0	≈5	203	2.2
7.0	60[*]	199	2.5
7.0	120	200	2.9
7.0	300[*]	199	2.7
7.0	1440[x]	197	6.2
8.0	≈5	203	0.58
8.0	60	205	20.6
8.0	120	201	23
9.0	≈5	195	0.77
9.0	60	193	42
9.0	120	197	54
10.0	≈5	197	2.2
10.0	60	198	86
10.0	120	197	112
10.9	≈5	200	14
11.0[#]	60	196	24
11.0[#]	120	197	47

---

1. Except where indicated, ± 0.1

[#] ± 0.2

2. Value is the mean of three or more experiments.

[x] One experiment.

[*] Mean of two experiments.

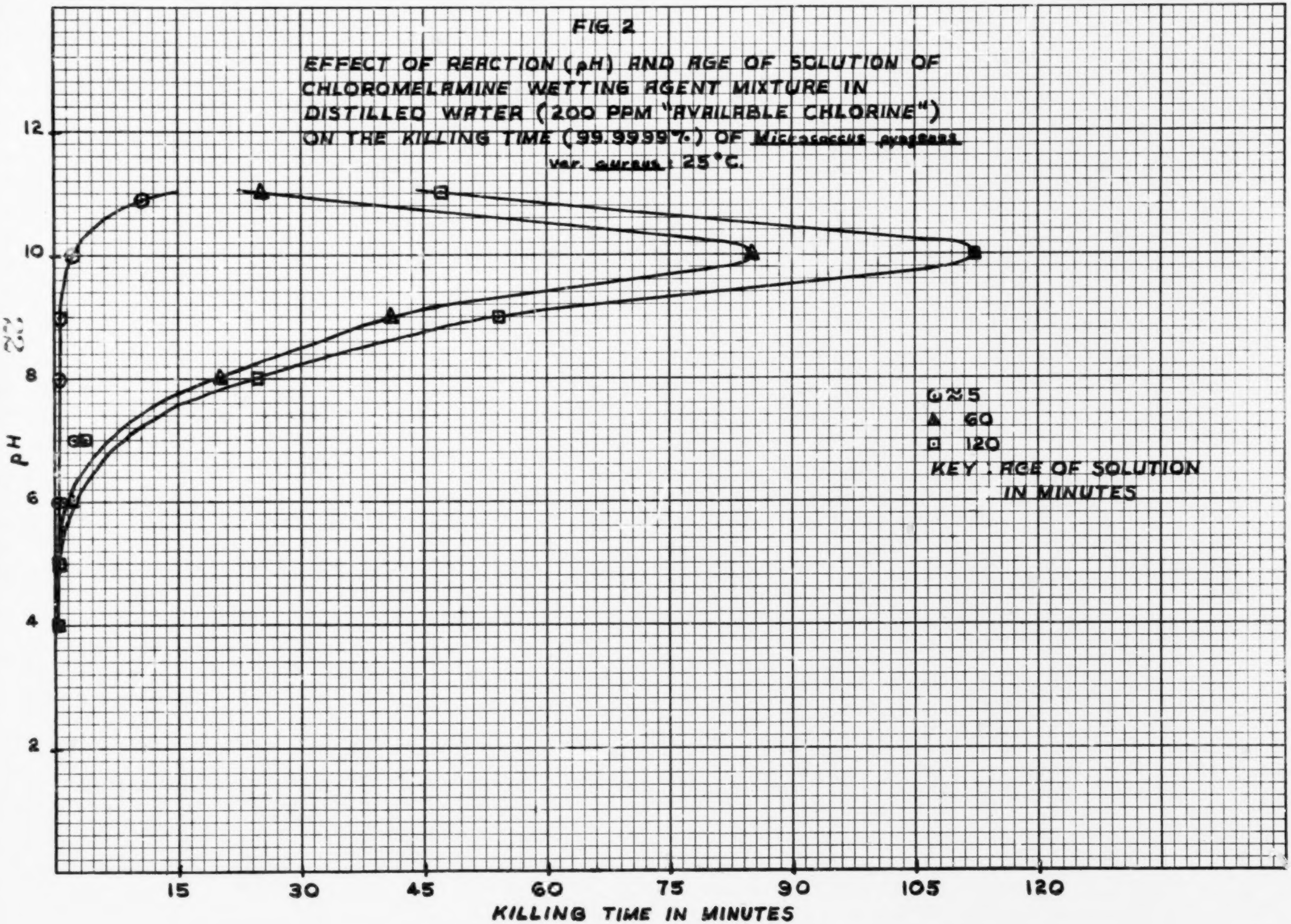


FIG. 2

EFFECT OF REACTION (pH) AND AGE OF SOLUTION OF CHLOROMELAMINE WETTING AGENT MIXTURE IN DISTILLED WATER (200 PPM "AVAILABLE CHLORINE") ON THE KILLING TIME (99.9999%) OF *Micrococcus pyogenes* var. *aureus* : 25°C.

TABLE IV

COMPARISON OF GERMICIDAL ACTIVITIES OF CHLOROMELAMINE 96P AND W.A.M. FORMULAE AT ABOUT pH 5

pH ± 0.1	Age of Solution in Min.	PPM Av. Cl.	Killing Time in Min.
Formula 96P Adjusted with Phosphate Buffer			
5.1	5	202	.22
5.1	5	202	< .17
Formula 96P Adjusted with Calcium Chloride and Sodium Bicarbonate			
4.6	5	202	.30
4.6	5	199	.25
W.A.M. Adjusted with Acetate Buffer			
5.0	5	201	.17
5.0	5	201	.13
5.0	5	200	.15

In Figure 2 the data from Table III are graphed with pH plotted against killing time in minutes. The data representing the Chloromelamine WAM solutions of about 5 minutes in age, show the effect of pH upon what is apparently chloromelamine unchanged or relatively unchanged as indicated by shorter killing times for solutions held at 5 minutes as compared with those held for 60 and 100 minutes. Only slight deviations in killing time occurred up to pH 10, but at pH 11 the killing time was lengthened to eleven minutes. For solutions held sixty minutes, there was apparently no significant increase in killing time over the solutions held 5 minutes between pH 4 and about pH 7. At pH 8 the killing time for the "60 minute" curve was about 21 minutes, at pH 9, 42 minutes, at pH 10, 86 minutes, but at pH 11 the killing time was only 24 minutes. Similarly, for the solutions held 120 minutes, the same general curve was shown but in each case the killing time was longer.

The killing times at pH 11 for the "60 minute" curve and the "120 minute" curve was shorter than for the corresponding curves at pH 10. No detectable effect of buffers upon the test organism in the absence of chloromelamine at each pH level was indicated in the periods observed. In the range of pH 10 and 11 this point was re-investigated following the observation of shorter killing times at pH 11 than at pH 10. With the buffer system used ($^{M}/160$ carbonate) no reduction in bacterial count was observed after two hours of exposure in the range of pH 10 and 11 at 25°C. in the absence of chloromelamine.

It is doubtful if natural waters would be as alkaline as some extremely high pH waters used, but many waters are encountered in the range of pH 8-9. Furthermore, it would not be uncommon for solutions to be held at least one hour in sanitizing mess gear. Under these conditions, the germicidal activity of a poorly buffered chloromelamine compound might be such as to render it unfit for use. The practical importance of compounds buffered to low pH values is evident.

4. Effect of Temperature

The effect of temperature on the germicidal activity of Chloromelamine Formula 96P in distilled water employing *Micrococcus pyogenes* var. *aureus* is recorded in Table V and shown graphically in Figure 3. To standardize solutions to a concentration of 200 ppm in studying only one variable (temperature) it was necessary to triturate samples due to lack of solubility, put them into solution and then adjust them to the required temperature. By dropping the temperature 10°C. the time required to kill 99.9999% of the test organism was doubled approximately.

In the second phase of the temperature study the special procedure developed by Morse and Feder and recommended by the National Research Council, Subcommittee on Food Supply(1) was used. In this study Chloromelamine Formula 96P (not triturated) was added directly to synthetic hard water. The germicidal activity was determined at 50°C. and 5°C. against *Escherichia coli* 198, A.T.C.C. #11229 and expressed as a killing time of 99.999% after solutions had been held 15 minutes and 120 minutes at the two temperatures (Table VI).

In the first test procedure the Formula 96P - $NaHCO_3$ and $CaCl_2$ solution was held at the test temperature for 10 minutes. The sterile peptone was added and the solution held an additional 5 and 110 minutes for a total holding time of 15 and 120 minutes before introducing the test organisms.

(1) Appendix: A Germicidal Rinse Compound For Field Use of the Armed Forces, Report to the Subcommittee on Food Supply of the Committee on Sanitary Engineering and Environment, National Research Council, 10 January 1952.

TABLE V

EFFECT OF TEMPERATURE OF CHLOROMELAMINE FORMULA 96P IN DISTILLED WATER ON THE KILLING TIME (99.9999%) Of *Micrococcus pyogenes* var. *aureus*: 200 ppm. av. cl.

Temperature in Degrees Centigrade	PPM Available Chlorine*	pH ± 0.1	Killing Time in Seconds*
50	201	3.0	< 10
35	202	3.0	< 10
25	190	3.0	6
15	196	3.0	13
5	201	3.0	28

* Mean of three experiments.

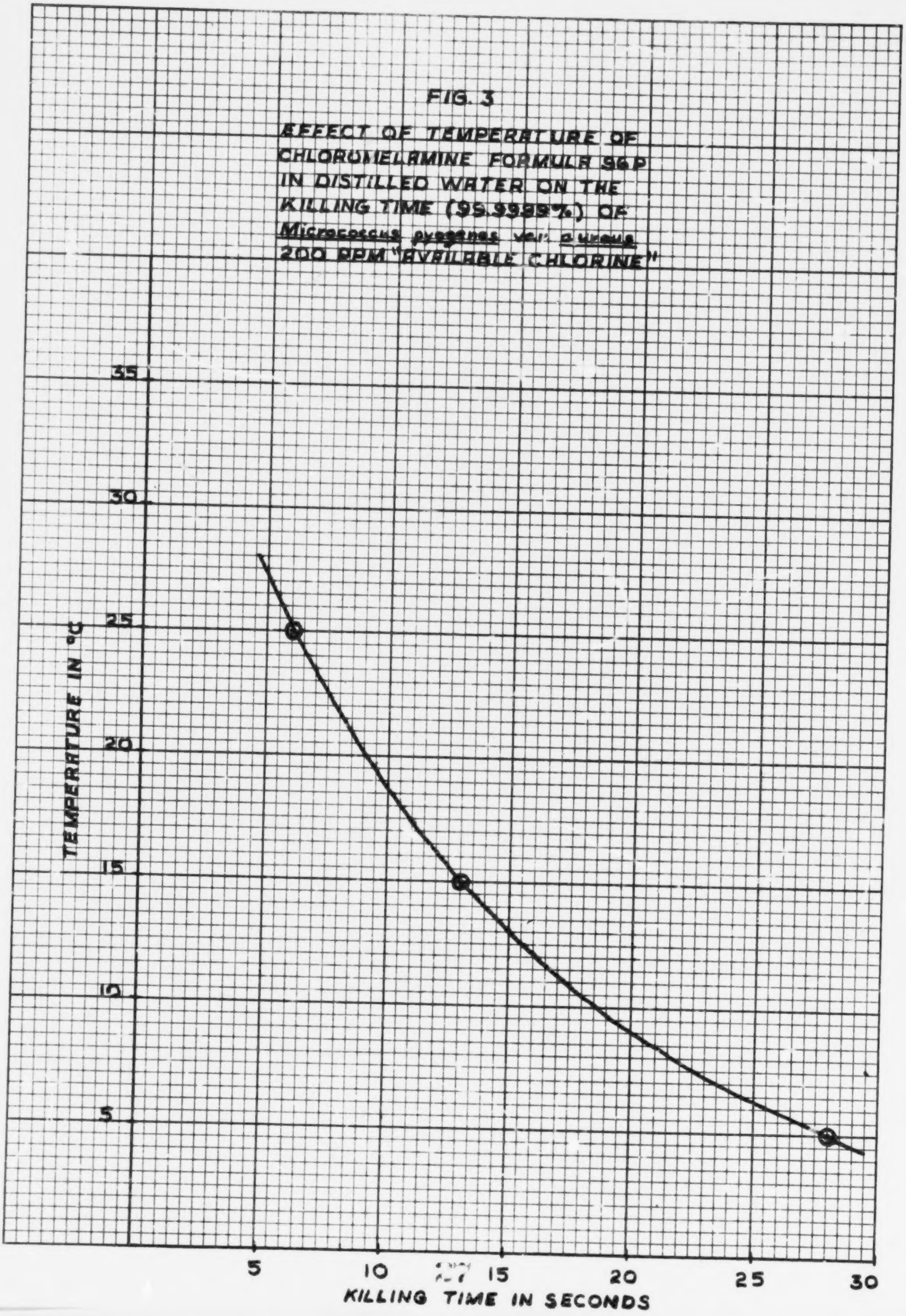


FIG. 3

EFFECT OF TEMPERATURE OF CHLOROMELAMINE FORMULA 96P IN DISTILLED WATER ON THE KILLING TIME (99.9999%) OF *Micrococcus pyogenes var. aureus* 200 PPM "AVAILABLE CHLORINE"

TABLE VI

EFFECT OF TEMPERATURE AND HOLDING TIME OF CHLOROMELAMINE FORMULA 96P IN SYNTHETIC WATER[1] ON KILLING TIME (99.999%) Of *Escherichia coli* (200 ppm. initial av. cl.)

Temp. °C.	Holding Time in Minutes	Peptone Contact (minutes)	pH ± 0.1	PPM Av. Cl.	Killing Time in Seconds
50	15	5*	4.8	161	< 10
50	120	110	4.9	59	60
5	15	5	4.8	176	306
5	120	110	4.9	170	264
50	15	15#	4.8	123	< 10
50	120	120	4.8	40	46
5	15	15	4.9	123	> 120
5	120	120	4.9	120	> 120

---

[1] 360 ppm. $CaCl_2$, 540 ppm. $NaHCO_3$, 2000 ppm. peptone.

* - Peptone introduced into Formula 96P solution 5 or 110 minutes before contact with test organism. Concentrations and killing times shown are the mean of three experiments.

- Peptone introduced into Formula 96P solution 15 minutes before contact with test organism. Concentrations and killing times shown are the mean of two experiments.

In the second test procedure the Formula 96P was in contact with the $NaHCO_3$, $CaCl_2$ and peptone for a full 15 and 120 minutes at the test temperature before introducing the culture. This procedure was used prior to the acceptance of the Morse and Feder method by the National Research Council. In all tests at 50°C. after holding the Formula 96P solutions 120 minutes, the concentration of available chlorine had dropped appreciably and the killing times were increased (Table VI).

In accordance with the specifications set up by the National Research Council a halogen-type germicide, to be approved for military use, should show by test a 99.999% reduction of Escherichia coli within 20 seconds when tested by the procedure reported herein. From the data in Table VI it is evident that Formula 96P does not meet these requirements.

5. Effect of Organic Matter

a. General.

The use of peptone as an organic soil has been employed by a number of investigators and recently by Morse and Feder*, in studying the effect of organic matter on halogen-type germicides. It was decided that a more-practical approach would be to employ a food soil similar to that actually used by the Armed Forces. To this end, a food soil was prepared, based on the U.S. Army field ration used during the early part of World War II with the formula as follows:

---

* Appendix: "A Germicidal Rinse Compound for Field Use of the Armed Forces, National Research Council, Subcommittee on Food Supply, 10 January 1952".

Meat	154.22	grams	Peanut Butter	1.09	grams
Eggs	29.02	"	Spinach	39.01	"
Milk	192.32	"	Carrots	39.01	"
Butter	18.14	"	Tomato juice	27.21	"
Oleomargerine	14.51	"	Orange juice	36.28	"
Sugar	58.05	"	Potatoes	123.37	"
Cereals	127.00	"	Beets	26.31	"
Beans	5.44	"	Corn	26.31	"
Peas	4.35	"	Applesauce	66.89	"
			Raisins	5.44	"

This food soil represented 14 classes of prepared foods (War Dept. Tech. Manual, TM 8-500) was mixed in the Waring blender, dispensed into paper cups and frozen in a deep freezer at -40°F. until required for tests. Just prior to each test, a sample of food soil was thawed and weighed into a sterile container.

Two phases of the basic studies employing organic matter were carried out. In the first,the concentration of Formula 96P was held constant at 200 ppm available chlorine and the concentration of food soil was varied from 1000 to 10,000 ppm. In the second, the food soil was held constant at 1000 ppm and the Formula 96P concentration was varied between 200 and 25 ppm available chlorine.

The general procedure employed in the basic studies to determine the effect of organic matter on the germicidal activity of Chloromelamine 96P was about the same as that used for other basic studies. A 100 ml. volumetric glass stoppered flask was tare-balanced and the food soil weighed directly into the flask. To this was added: $CaCl_2$*, distilled water to prevent precipitation, $NaHCO_3$, sufficient standardized chloromelamine solution to give the desired concentration of available chlorine, and sufficient distilled water to bring the solution to volume. All concentrations were doubled in accordance with the procedure employed. After the chloromelamine was mixed with the fool soil and other materials, the mixture was permitted to stand 15 minutes, after which 5 ml. of the double-strength germicide was introduced into the medication tube containing the 5 ml. of double-strength bacterial suspension and the germicidal activity tests were carried out in the usual manner.

---

* Based on synthetic hard water described in: "A Germicidal Rinse Compound for Field Use of the Armed Forces, National Research Council, Subcommittee on Food Supply, 10 January 1952". (To give 360 ppm $CaCl_2$ and 540 ppm $NaHCO_3$ in medication tube).

b. Results.

In the first phase of this study the concentration of Chloromelamine 96P was adjusted to approximately 200 ppm available chlorine and the food soil was varied from 1000 ppm to 10,000 ppm. In Table VII it may be noted that with a food soil of 1000 ppm the initial available chlorine concentration made after the germicide and food soil had been mixed for 15 minutes was 196 ppm and the killing time was 0.41 minutes. When the food soil was increased five-fold to 5,000 ppm the available chlorine dropped to 180 ppm and the killing time was increased to 1.4 minutes. With 10,000 ppm food soil the available chlorine initial concentration was 173 ppm and the final concentration was 161 ppm with a killing time prolonged to 3.9 minutes.

The five-fold increase in food soil from 1,000 to 5,000 ppm increased the killing time 3.4 times. Doubling the food soil concentration from 5,000 to 10,000 ppm increased the killing time 2.8 times its former value.

In the second phase of this study, the food soil was maintained at 1,000 ppm and the concentration of available chlorine was varied from 200 ppm down to 25 ppm. In Table VIII it may be noted that with a concentration of about 200 ppm available chlorine, the killing time was 0.41 minutes; at 100 ppm the

TABLE VII

EFFECT OF VARYING CONCENTRATIONS OF "ORGANIC SOIL"[1] IN 200 PPM AVAILABLE CHLORINE CHLOROMELAMINE 96P; 25°C.[2]

PPM Food Soil	PPM Available Chlorine Initial	Final	pH	Kill Time in Minutes 99.9999%
1,000	196†	‡	4.9 ± .15	.41
5,000*	180	176	4.8	1.4
10,000*	173	161	4.9	3.9

* One experiment.
† Mean of 4 experiments.
‡ Kill time short, final titration not made.
1. Based on U.S. Army Field Ration
2. Synthetic hard water: 360 ppm $CaCl_2$; 540 ppm $NaHCO_3$.

TABLE VIII

EFFECT OF VARYING CONCENTRATIONS OF "AVAILABLE CHLORINE" FROM CHLOROMELAMINE 96P IN 1,000 PPM FOOD SOIL[1]; 25°C.[2]

Theoretical Use PPM Available Chlorine	PPM Available Chlorine		pH ± .15	Killing Time in Minutes 99.9999%
	Initial	Final		
200	196	‡	4.9	.41ǂ
100	102	105	5.9	4.9ǂǂ
50	50	47	6.5	25ǂǂ
25	25	24	7.3	>30*

1. Based on U.S. Army Field Ration.
2. Synthetic hard water; 360 ppm $CaCl_2$; 540 ppm $NaHCO_3$.

ǂ Mean of 4 experiments.

ǂǂ Mean of 3 experiments.

* Two experiments.

‡ Kill time short; final titration not made.

killing time was 4.9 minutes; at 50 ppm the killing time was 25 minutes and at 25 ppm available chlorine very little reduction in bacteria had occurred at 30 minutes. Increasing the concentration of available chlorine to twice its value, i.e., from 50 ppm available chlorine up to 100 ppm decreased the killing time to 1|5 its value. A further doubling in the concentration of available chlorine from 100 ppm to 200 ppm available chlorine reduced the killing time to less than 1|10 its previous value. Comparing these trends with the study of the effect of concentration in Table I using synthetic water in the presence of peptone it may be observed that somewhat similar behavior is illustrated. In the absence of organic matter in distilled water (Table I) the effect of doubling the concentration has much less effect on shortening the killing time.

6. Basic Studies

Conclusions

Basic studies on the effect of concentration, reaction, temperature and organic matter on the germicidal activity of Chloromelamine Formula 96P were conducted.

The general conclusions reached were as follows:

(1) Solutions of chloromelamine must be well buffered at low pH levels in order to maintain effective germicidal activity for periods of time comparable to those used for disinfection of mess gear. In a poorly buffered formulation of chloromelamine (Wetting Agent Mixture), the pH increased as the concentration decreased, resulting in loss of bactericidal activity.

(2) With Formula 96P in distilled water at 25°C., killing times were approximately doubled by halving the available chlorine concentration. When tested in a synthetic hard water containing peptone the times required to kill 99.9999% of the test cultures were increased markedly.

(3) The killing times were increased when chloromelamine solutions were buffered to alkaline reactions. Great variations in killing time occurred with alkaline solutions of chloromelamine depending on the time elapsing between addition of the buffer to adjust pH and the killing time determination.

(4) Chloromelamine Formula 96P when held at 5° and 50°C. for 15 and 120 minutes in a synthetic hard water containing peptone and tested by the procedure approved by the National Research Council, failed to kill 99.999% of the test organism in 20 seconds.

A food soil based on the U.S. Army field ration in a concentration of 1000 ppm with a concentration of Formula 96P of 200 ppm available chlorine resulted in a killing time (99.9999%) of 0.41 minutes. This killing time was not grossly different from that which was found for 200 ppm available chlorine of chloromelamine at a comparable pH (pH 5-7) in the absence of organic matter. However, when the concentration of food soil was increased to 5000 and 10,000 ppm with the concentration of chloromelamine remaining constant, there was a significant increase in the killing time. Likewise, when the concentration of food soil was held constant at 1000 ppm and the concentration of available chlorine dropped to 100, 50 and 25 ppm there was a marked increase in killing time.

From the data available in this study it appeared that for a concentration of 1000 ppm food soil at 25°C. a minimum concentration of 200 ppm of available chlorine from Formula 96P produced fairly efficient germicidal activity but that a concentration of slightly higher than 200 ppm was desirable.

Part C

Stability Studies

General

The stability of Chloromelamine 96P was studied under a wide range of temperature and humidity conditions and with three types of containers (brown glass bottle, plastic bag, cardboard box). A number of samples of Formula 96P were placed in storage at Wright-Patterson Air Force Base (Equipment Laboratory) and in Cincinnati (Environmental Health Center, Faller Building) so that samples could be withdrawn periodically and studied for bactericidal activity and by chemical tests. A planned schedule covering a two year period was prepared and established in such a manner that a greater number of samples could be withdrawn from environments where deterioration was at a high rate.

The directions for use of the packaged chloromelamine formulations as set forth by the Quartermaster General specify the use of one package (3.35 oz.) per 25 gallons of water. To simulate these conditions it was necessary to weigh a sample of the Formula 96P (after a definite period of deterioration), dissolve it in distilled water (in a concentration equal to one package per 25 gallons of water), and determine the germicidal activity by the survival-curve procedure. Standardization of the solution by titration would be erroneous because this would maintain the available chlorine concentration at a constant level, whereas in reality this does not necessarily occur. Standardization by weight simulates actual "use" conditions. However, the moisture concentration of the samples under deterioration study was constantly increasing (particularly in the samples stored under conditions of high humidity). Moisture determinations were made on each sample prior to making a bactericidal test in order that the quantity of sample to be weighed could be corrected for moisture absorption.

One of the purported properties of chloromelamine is that it releases its active component slowly, because of its limited solubility, thus maintaining a reserve available for disinfection even in the presence of organic matter. For stability samples where only a limited number of tests could be made it was necessary to triturate each sample to expedite solution to get a mean value for germicidal activity from one test rather than to run a series of tests with progressing time as the compound dissolved slowly.

Results

Formula 96P which had not been exposed to deterioration conditioning, diluted to a concentration equal to one package (3.35 oz.) to 25 gallons of water, when triturated and prepared in distilled water showed a mean chloromelamine concentration of 187 ppm available chlorine with a pH of 3.0. The mean killing time (99.9999%) for Micrococcus pyogenes var. aureus was 0.14 (about 8 seconds). This mean killing time was used as a "base line" or "yardstick" by which deterioration might be measured.

Table IX is a composite of values of all stability studies. The "base line" storage time is entered in this table under "Time of Storage in Days" as zero. The mean concentration of available chlorine in a concentration equivalent to 3.35 oz. per 25 gallons of water was 187 ppm available chlorine with a pH of about 3.0.

160°F., 90 to 100% R.H.

Samples stored at 160°F. with 90 to 100% relative humidity (Table IX) were the first to show the effects of storage conditions. Samples packaged in cardboard boxes completely broke down after only two hours of exposure, evolving what apparently was chlorine gas. The resulting mass was charred and Formula 96P changed into a dry, burned, gingerbread-appearing material. No available chlorine was demonstrated in the samples. Survivor curves showed that in spite of the absence of titratable chlorine, the material showed a killing time of 7.2 minutes. By previous tests Micrococcus pyogenes var. aureus proved to be relatively stable to acid or alkali under the conditions of these experiments, from pH 4 to pH 11. However, buffers in the range of pH 3.0 had a definite killing effect upon the organism and indications were that the killing noted in the deteriorated product was primarily due to the effect of the buffer.

Formula 96P packaged in plastic bags remained in usable condition for only 1.2 days under the conditions of high temperature and high humidity. The plastic bags had completely deteriorated and the contents had turned brown after this exposure period (1.2 days). Only one bag remained intact and its contents were studied for germicidal activity. The available chlorine concentration showed a mean of 108 ppm, a pH of about 3.1 and a mean killing time of 25 seconds. Of the entire lot of samples packaged in plastic bags and stored under these test conditions, only one sample was usable.

The samples in brown glass bottles were covered with plastic screw caps. These softened under the conditions of high temperature and high humidity resulting in absorption of moisture. Within 28 hours (1.2 days) Formula 96P deteriorated sufficiently so that the mean concentration of available chlorine was only 81 ppm, the pH was about 3.1 and the killing time had extended to a mean of 46 seconds. Formula 96P, after this exposure period, was composed of grey, moist lumps.

TABLE IX

EFFECT OF ENVIRONMENT OF CHLOROMELAMINE FORMULA 96P ON KILLING TIME (99.9999%) Of *Micrococcus pyogenes* var. *aureus*: 25°C.

		BOTTLE			BAG			BOX		
Storage Conditions of Sample	Time of Storage in Days	pH$^{\#}$	PPM Available Chlorine^{1}	Killing Time in Seconds^{1}	pH$^{\#}$	PPM Available Chlorine^{1}	Killing Time in Seconds^{1}	pH$^{\#}$	PPM Available Chlorine^{1}	Killing Time in Seconds^{1}
	0	3.0	187	8	3.0	187	8	3.0	187	8
160°F. 90-100% RH	0.08							3.1	0	432
	1.2	3.1	81	46	3.1	108	25			
	27	3.0	0	186						
160°F. 6% RH	14	3.0	133	15	3.0	140	14	3.1	86	31
	77	3.0	68	57	3.0	114	17	3.0^{x}	58	47
	112	3.0	69	66	3.0	88	44	3.2	15	153
	174	3.0	52	70	3.1	42	118	3.1	17	177
Room Temp.^{2}	51	3.0	172	10	3.0	186	11	3.0	176	11*
	97	3.0	186	8*	2.9	183	13*	3.0^{x}	182	10*
	146	3.0	190	< 30	3.0	205	< 30	3.0	165	< 30
	194	3.0	163	< 30	3.0	167	< 30	3.0	169	< 30
32°F.^{3}	16	3.0	181	9	3.0	178	11*	3.0	169	11
	78	2.9	179	8	3.0	192	8	3.0^{x}	189	11
	113	3.0	204	< 30	3.0	227	< 30	3.0	189	< 30
	175	3.0	181	< 30	3.0	191	< 30	3.0	177	< 30
-65°F.^{3}	16	3.0	170	8	3.0	170	8	3.0	168	9
	78	3.0^{x}	179	11	3.0	182	14	2.9	185	11
	113	3.0	192	< 30	3.0	182	< 30	3.0	209	< 30
	175	3.0	180	< 30	3.0	194	< 30	3.0	197	< 30

1. Each value is the mean of 3 or more experiments except where indicated otherwise.
2. Approximately 75°F. and uncontrolled R.H.
3. Uncontrolled R.H.

* - Mean of two experiments.
# - ± 0.1 unless otherwise indicated.
x - ± 0.2

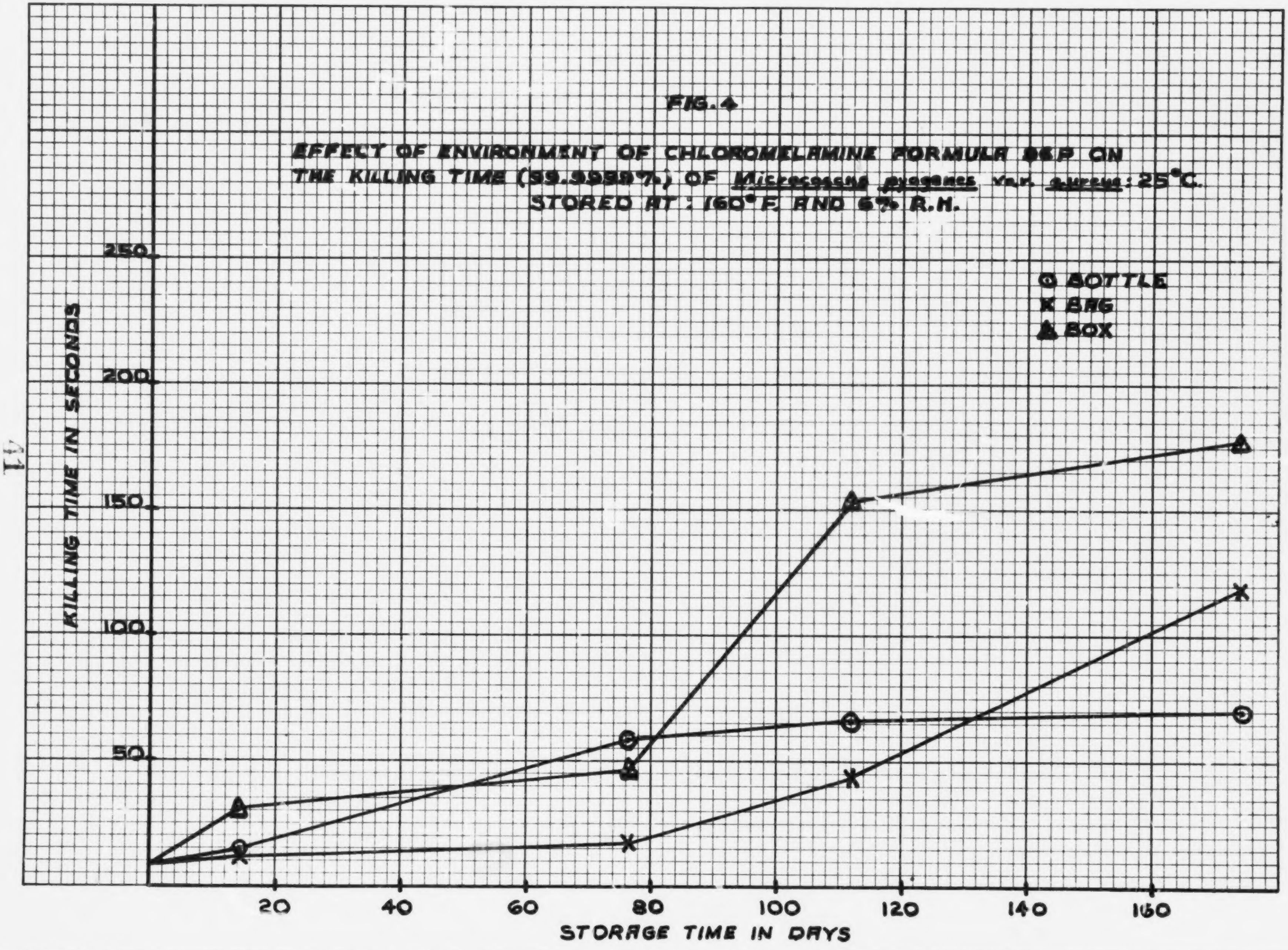


FIG. 4

EFFECT OF ENVIRONMENT OF CHLOROMELAMINE FORMULA 86P ON THE KILLING TIME (99.9999%) OF *Micrococcus pyogenes* var. *aureus*: 25°C. STORED AT: 160°F. AND 6% R.H.

After exposure of bottle samples 27 days, there was no titratable chlorine. The pH was unchanged and no chlorine was detectable, but there was still some germicidal activity. In one experiment, the killing time was 186 seconds. However, the material was definitely not usable at this stage. It was grey, moist and had the consistency of putty.

160°F. 6% R.H.

Table IX, and Figure 4 show data for stability studies carried out at 160°F. and 6% relative humidity. The samples packaged in brown bottles stored under these conditions for 14, 77, 112 and 174 days had a pH of about 3.0 and killing times of 15, 57, 66 and 70 seconds respectively. At these storage conditions the samples in plastic bags held for 14, 77, 112 and 174 days had a pH value of about 3.0 and mean killing times of 14, 17, 44 and 118 seconds respectively. The samples in cardboard boxes held for 14 days at 160°F. and 6% relative humidity showed values of 86 ppm available chlorine and 31 seconds killing time; at 77 days, 68 ppm available chlorine and 47 seconds killing time; at 112 days, 15 ppm available chlorine, 153 seconds killing time; and at 174 days, 17 ppm available chlorine, 177 seconds killing time. The pH values for all samples were about 3.0. From these data it appeared that at 160°F. and 6% relative humidity, Formula 96P lost appreciable activity in each type of container but this deterioration was greatest in samples stored in cardboard boxes.

Room Temperature (about 75°C.) Uncontrolled R.H.

Samples stored at room temperature (about 75°F.) in all three types of containers appeared to have lost little of their germicidal activity after 194 days of storage. Actual killing times were not determined at 194 days, but a test was made to show that the killing time had not extended to as much as 30 seconds. Data are presented in Table IX.

32°F. Uncontrolled R.H.

Data for stability studies at 32°F. with uncontrolled relative humidity are tabulated in Table IX. After storage for 175 days, tests were made to show that the killing time had not progressed beyond 30 seconds. There was no indication that any significant changes had occurred in these samples and it is probable that killing times were about the same as for the "base line" determination.

-65°F. Uncontrolled R.H.

Indications are that at this low temperature (-65°F.) no significant changes had occurred in any of the three types of containers after 175 days (Table IX).

On June 30, 1952 all samples of Formula 96P were removed from storage chambers at Wright-Patterson Air Base and stability studies discontinued. Samples at Wright-Patterson Air Base had been under storage for about 270 days and those stored at room temperature at the Environmental Health Center, Faller Building, had been held for 294 days. After 270 days of storage, 160°F., 6% R.H. samples in bottles were granular at the surface but caked in the bottom; samples in plastic bags were intact but had a charred appearance and were very lumpy; box samples were lumpy and brown and the boxes were very fragile. Samples held at room temperature in bottles for 294 days were granular while the plastic bags were intact but the contents lumpy and the cardboard boxes were fragile with the formulation moist and lumpy. Samples held at 32°F. all were intact but those samples held in plastic bags and boxes were lumpy. At -65°F. all containers were intact. Samples in bottles and plastic bags showed little if any change in physical properties. Those samples in boxes were slightly moist and lumpy.

Available Chlorine as a Measure of Germicidal Activity in Stored Samples

In Table I are presented data showing killing times of concentrations of Chloromelamine Formula 96P which theoretically should yield 200, 100, 50, 25, and 12.5 ppm available chlorine. Killing times ranged from 0.10 minutes (6 seconds) for the higher concentration (actual concentration of 190 ppm available chlorine) to 0.93 minutes (56 seconds) for 12.5 ppm. With half the available chlorine concentration, the killing time was extended to an average of about 1.8 times. Data from Table I are presented graphically in Figures 5-A, 5-B and 5-C as "fresh samples", to be used for comparison with stored samples.

In Figure 5-A are plotted data taken from both Tables I and II. The curve for stored samples represents samples packaged in brown glass bottles under the varied conditions of environment as shown in Table II. The available chlorine concentration was plotted against killing time. It appeared that as the concentration of available chlorine dropped in the stored samples, the available chlorine concentration became an increasingly poorer measure of germicidal efficiency. It is evident in Figure 5-A that with a concentration of 50 ppm available chlorine, the killing time was less than 25 seconds or greater than 70 seconds, depending upon the "history" of the sample.

Figures 5-B and 5-C show similar data comparing fresh samples with samples stored in plastic bags and cardboard boxes respectively. The general trends illustrated for the stored bottle samples held for the samples stored in plastic bags and cardboard boxes insofar as deterioration had progressed.

These data demonstrate clearly the need for a field chemical test which measures more accurately the germicidal activity of the available chlorine present.

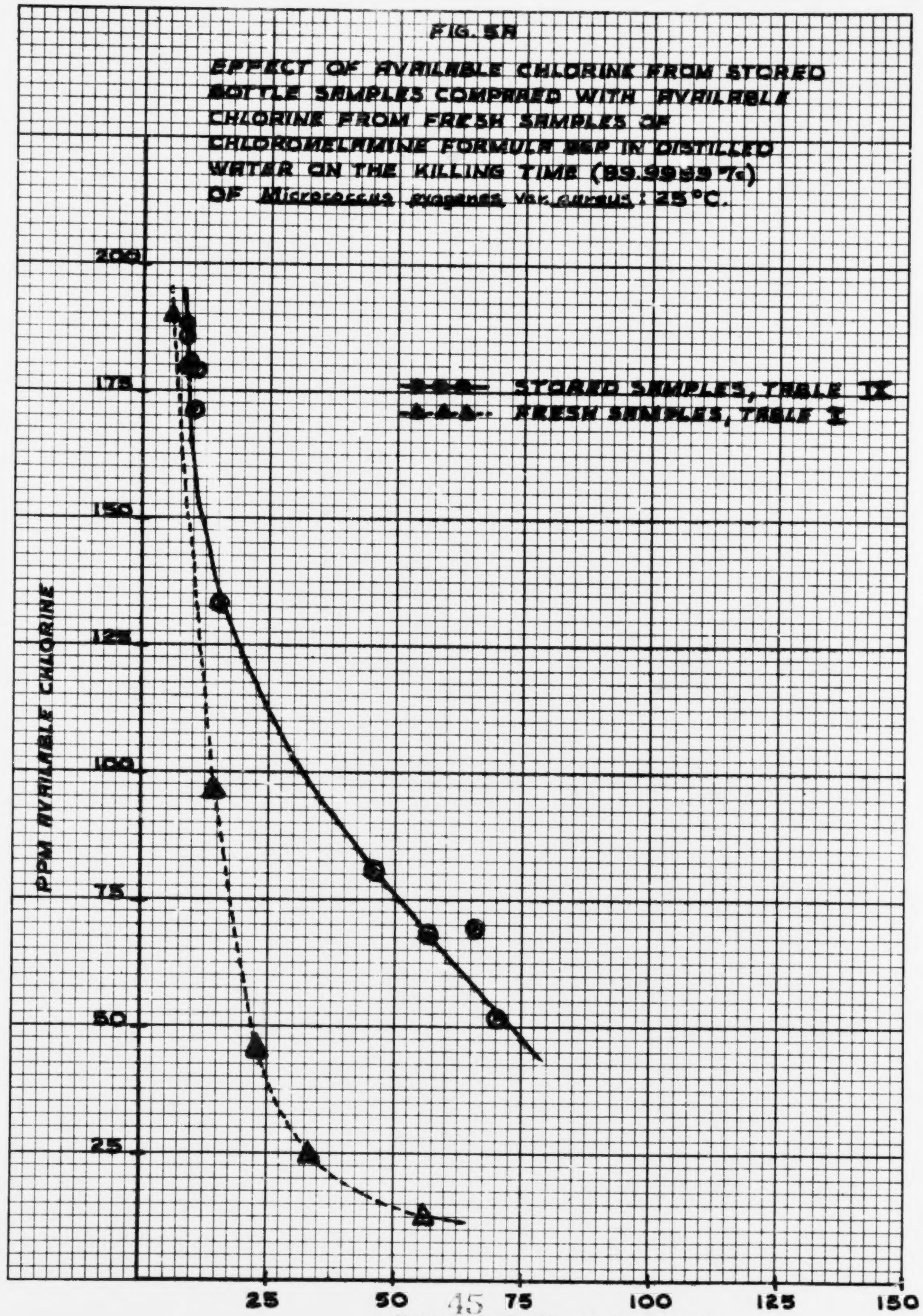


FIG. 5A

EFFECT OF AVAILABLE CHLORINE FROM STORED BOTTLE SAMPLES COMPARED WITH AVAILABLE CHLORINE FROM FRESH SAMPLES OF CHLOROMELAMINE FORMULA 88P IN DISTILLED WATER ON THE KILLING TIME (99.999 %) OF *Micrococcus pyogenes* var. *aureus* : 25°C.

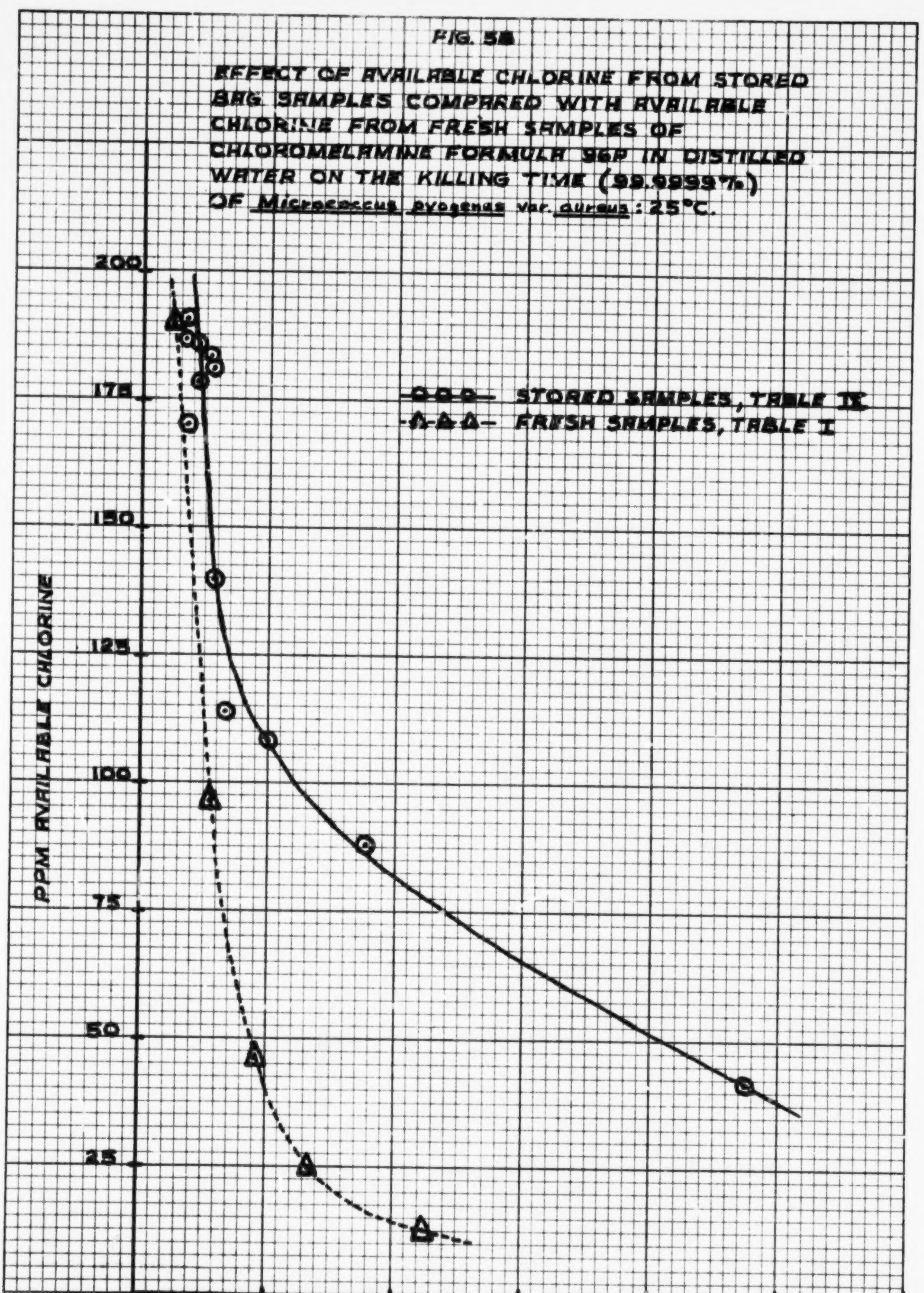


FIG. 5B

EFFECT OF AVAILABLE CHLORINE FROM STORED BAG SAMPLES COMPARED WITH AVAILABLE CHLORINE FROM FRESH SAMPLES OF CHLOROMELAMINE FORMULA 96P IN DISTILLED WATER ON THE KILLING TIME (99.9999%) OF *Micrococcus pyogenes* var. *aureus*: 25°C.

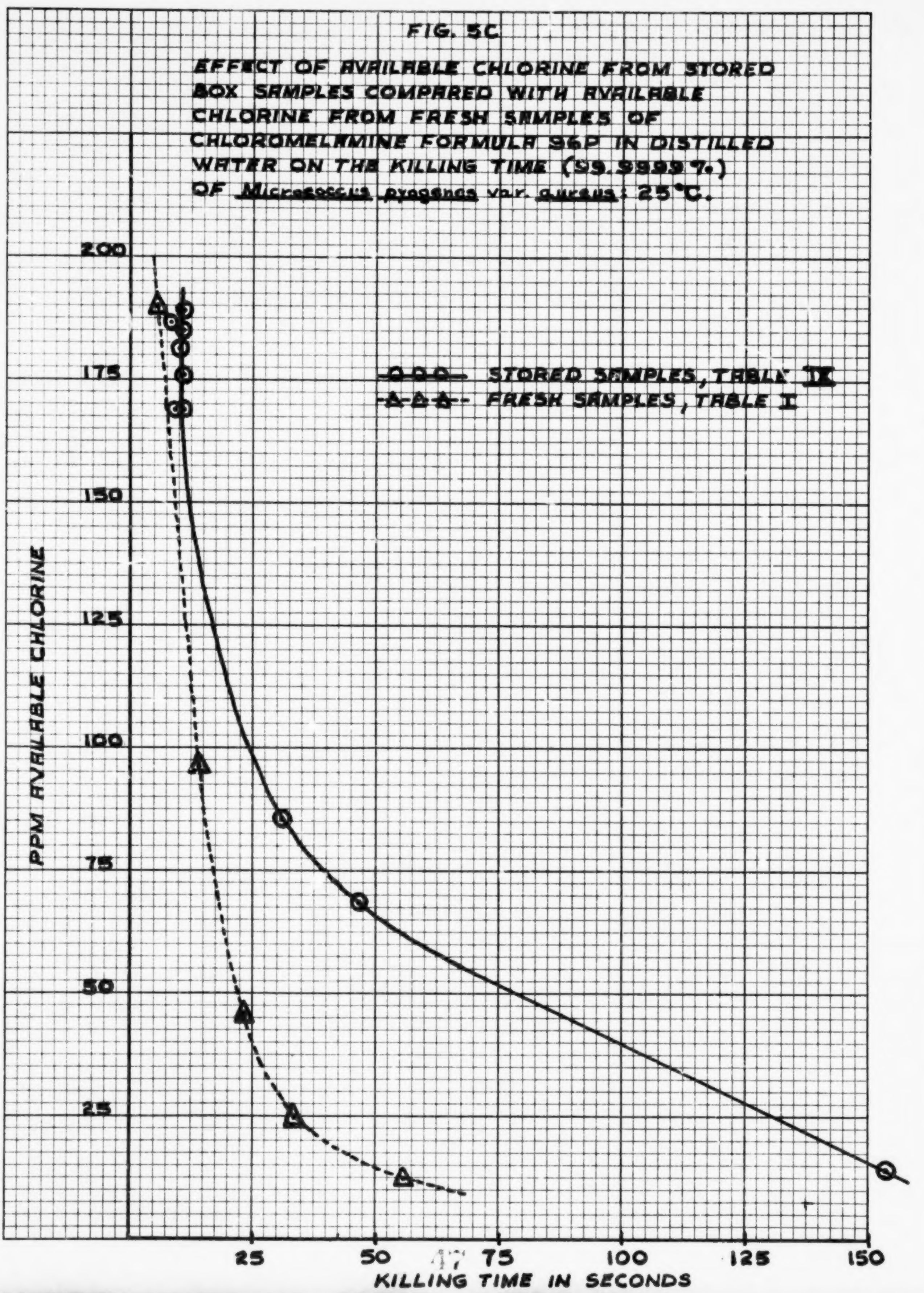


FIG. 5C

EFFECT OF AVAILABLE CHLORINE FROM STORED BOX SAMPLES COMPARED WITH AVAILABLE CHLORINE FROM FRESH SAMPLES OF CHLOROMELAMINE FORMULA 96P IN DISTILLED WATER ON THE KILLING TIME (99.999%) OF *Micrococcus pyogenes* var. *aureus*: 25°C.

Conclusions

(1). Storage at temperatures above 75°F., especially in the range of 160°F., caused complete deterioration in a matter of hours or days if the humidity was high. Deterioration was slower if the relative humidity was lower (6%) and the plastic bag appeared to be the best of the three types of containers studied. Packaging studies to improve the storage life at high temperatures and humidity are indicated.

(2). Little, if any, deterioration appeared to occur at temperatures of 75°F. or below (uncontrolled humidity) over a period of 175 to 194 days.

(3). Available chlorine as measured by sodium thiosulphate-starch iodide titration appeared to be a satisfactory measure of the germicidal activity of fresh chloromelamine samples and of stored samples if the residual was near the initial concentration. However, in stored samples where a drop in available chlorine was evidenced, available chlorine as measured by this procedure was a poor index of germicidal activity.

Part D

Disinfection of Mess Gear

The results are presented in Table XIX. The data show that the hypochlorous acid solution was fairly stable at a concentration of 200 ppm available chlorine but the Chloromelamine MQ solution of 0.1 per cent by weight lost available chlorine rapidly at first and more slowly on prolonged contact. Over 10 per cent of the observed available chlorine was lost in one hour, and nearly 20 per cent was lost in two hours. The citric acid at a concentration of 374 ppm had a rapid hypochlorous acid-chlorine demand amounting to 0.44 mg. Cl_2 per milligram of citric acid in a two hour period under the conditions of the test. The addition of 215 ppm citric acid to the 374 ppm already in the chloromelamine formula increased the loss of available chlorine by a small percentage. Peptone in a concentration of 1,000 ppm had a very rapid demand for free chlorine as observed from the loss of chlorine in column 6, Table XIX. On the other hand, the reduction of available chlorine from Chloromelamine MQ occurred at a much lower rate in the presence of 1,000 ppm peptone. After two hours, 58 per cent of the available chlorine had been dissipated from the hypochlorous acid solution compared to a 23 per cent loss in the Chloromelamine MQ. Additional data on the stability of these chloromelamine products in substrates containing peptone and sulframin are presented in Table XX. Both available chlorine and the free chlorine were determined periodically. The results show that:

(1) Although there is a progressive loss of available chlorine from a 55-5 chloromelamine in peptone solution, it is more significant from the standpoint of disinfection that the free chlorine was entirely consumed by the peptone during the 15 to 30 minute period after the peptone was added. On prolonged contact, the data indicate that free chlorine reappeared at concentrations varying from 0.1 to 0.6 ppm.

General

Following the basic studies in which the comparative germicidal activity of Chloromelamine 96P was studied, applied studies were set up to demonstrate the germicidal efficiency of Chloromelamine 96P for the disinfection of mess gear. The test organism Micrococcus pyogenes var. aureus was used to represent Gram positive organisms commonly found on food utensils. The number of test organisms per utensil was adjusted to be in the range of a standard swab count of one million. This count was selected after a review of food utensil swab counts made in the U.S. Public Health Service mobile laboratories during World War II. The highest swab counts reported were in the range of one million organisms per utensil. Studies carried out prior to the chloromelamine project, revealed that a drinking glass dipped in a suspension of about 100 million bacteria per ml., and drained, had a swab count of about 1 million bacteria. The swabbing surface of a meat can without cover approximates that of a drinking glass. A swab count after disinfection must not exceed 100 organisms per utensil under A.P.H.A. standards. To achieve the reduction of approximately one million organisms per utensil initial count to 100 per utensil after disinfection required 99.99% bacterial destruction. In the basic studies on chloromelamine, the end point of 99.9999% destruction was selected. The basic studies were of a more-precise nature and the selection of an end point to the fourth decimal place is justified, based on checking of replicate determinations. In the disinfection of mess gear, there are certain factors not as easily controlled as in the basic studies. The end point of 99.99% elimination of exposed bacteria was taken as an end point in the disinfection of mess gear with an initial inoculum of one million or more per utensil. It would appear that a germicide solution capable of reducing the viable organisms from about one million (or more) to about 100 per utensil (99.99% reduction) would provide sufficient germicidal activity for most conditions provided other properties of the germicide were satisfactory. It is probable that under the conditions of actual use mess gear would have considerably less than one million per ml. of swab rinse.

The food soil employed, based on the U.S. Army field ration during World War II, was set at a concentration of 0.1% in the inoculum, wash, rinse 1 and rinse 2. Synthetic hard water containing calcium chloride and sodium bicarbonate was used as prescribed in the National Research Council Subcommittee on Food Supply report entitled: "A Germicidal Rinse Compound for Field Use of the Armed Forces", 10 Jan. 1952. (pages 1 and 2).

A 15-second brushing in the wash, followed by two 5-second rinses in each germicide rinse was considered to be an approximation of minimum exposure times under field conditions.

Procedure

Twenty sets of meat cans with spoon and fork inclosed were wrapped in brown paper and sterilized in the autoclave. At the beginning of an experiment the wrappers were removed. The meat cans without cover with spoons and forks attached to handle were inoculated by dipping in a suspension of Micrococcus pyogenes var. aureus having a plate count of approximately 100 million colonies per ml. and containing 0.1% food soil*. Following inoculation, utensils were hung on a wire line and drained for 1 hour at room temperature (about 27°C.).

---

* As used in basic studies

Three 4-gallon galvanized iron cans were prepared for the wash, rinse 1 and rinse 2. Each can was sterilized in the autoclave and 10 liters of sterile distilled water (previously adjusted to desired temperature) introduced. Fifteen minutes prior to the start of the disinfecting tests, salts, detergent, and food soil based on U.S. Army field rations were added to the G.I. can used for washing. Salts, germicide and food soil were added to the second and third G.I. cans for rinses 1 and 2. All solutions were stirred and permitted to stand 15 minutes at which time meat cans without cover with spoon and fork attached were washed, rinsed, drained 1 minute and swabbed. Meat cans without cover, spoons and forks were brushed for 15 seconds in the wash followed by rinsing for 5 seconds (or longer in some instances) in each of the two rinses. Rinsing was accomplished with constant agitation by raising and lowering the mess gear during the exposure period. The swab test was carried out according to Standard Methods as described by the American Public Health Association, Subcommittee on Food Utensil Sanitation. Each swab contained 4 ml. of buffered solution with sufficient sodium sulfite for neutralizing the residual chlorine from the germicide rinses. Four similar utensils comprised a sample to be swabbed in one vial e.g., four forks, four spoons or four meat cans.

Following the swabbing, swab sticks were burned in-two on a hot wire, solutions were shaken as a prescribed in the standard procedure and appropriate dilutions made (generally higher than required in the standard) followed by plating with tryptone glucose extract agar. Incubation of plates was at 35°C. for 48 hours.

Preliminary studies:

In this study it was desirable to determine the length of time the disinfectant continued to act after removal of gear from rinse waters. This information was necessary to the selection of the holding time between disinfection and swabbing of utensils. To this end, an experiment was set up in which inoculated mess gear (meat can minus cover, spoon and fork) were dipped for 5 seconds in 0.3% Sulframin AB, rinsed for 5 seconds in each of two solutions of approximately 187 ppm available chlorine of Chloromelamine 96P (7-8°C.) drained at room temperature for 1, 10, 30 or 120 minutes and swabbed. Control determinations indicated that untreated utensils held for 120 minutes in air showed a reduction of about 60%. Results for treated utensils are presented in Table X. Although each value represents only one experiment, the general trend indicates slight changes in the percentage reduction between 1 and 120 minute holding time. After a holding period of 120 minutes complete disinfection did not occur. The results indicate that slight disinfection continued for about 30 minutes after the utensils were dipped.

Results

In Table XI, line 1 it is of interest to note that the physical removal of bacteria by brushing meat cans without cover for 15 seconds in 0.3% Sulframin AB and rinsing for 5 seconds in each of two solutions containing .012% Sulframin AB (calculated to be the same concentration as contained in a solution containing 187 ppm available chlorine of Chloromelamine Formula 96P) resulted in a reduction of 87.30% of the bacteria. Thus, even in the absence of chloromelamine, an appreciable reduction in swab count resulted. Indications are that this

TABLE X

PERCENT REDUCTION OF *Micrococcus pyogenes* var. *aureus* ON MESS GEAR DURING STORAGE AT ROOM TEMPERATURE FOLLOWING RINSING IN CHLOROMELAMINE 96P*

	Storage Times (Min.) at 27°C. after 5 sec. wash in 0.3% Sulframin and 5 sec. rinse in 2 solutions of about 187 ppm av. cl. Chloromelamine 96P: 7-8°C.			
	1	10	30	120
Meat Cans without covers	98.80	99.99	99.97	99.98
Spoons	99.57	99.70	99.88	99.90
Forks	99.20	99.40	99.90	99.90

* Each value is from one experiment only, based on 4 utensils.

TABLE XI

PERCENTAGE REDUCTION OF *Micrococcus pyogenes* var. *aureus* ON MEAT CAN AFTER TREATMENT

		Temp. °C.	pH Wash	Rinse No. 1	Rinse No. 2	Exposure (Seconds) In Each Rinse*				
						5	15	60	120	300
I	15 Sec. Brushing Sulframin AB** No Germicide	6	8.0 ±0.3	pH: 7.9±0.2 ppm Av. Cl. Init.: - Final: -	pH: 7.9±0.2 ppm Av. Cl. Init.: - Final: -	87.30	-	-	98.25	98.00
II	15 Sec. Brushing 50 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	7	10.5 ±0.1	pH: 8.1 ppm Av. Cl. Init.: 38 Final: 25	pH: 7.9 ppm Av. Cl. Init.: 38 Final: 27	98.85	-	-	99.94	>99.99
III	15 Sec. Brushing 187 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	8	10.6 ±0.1	pH: 8.5±0.1 ppm Av. Cl. Init.: 164 Final: 147	pH: 8.3±0.1 ppm Av. Cl. Init.: 168 Final: 153	>99.99	-	>99.99	>99.99	-
IV	15 Sec. Brushing 187 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	24	10.5 ±0.1	pH: 8.0±0.1 ppm Av. Cl. Init.: 166 Final: 121	pH: 8.0±0.1 ppm Av. Cl. Init.: 166 Final: 128	>99.99	-	100.00	100.00	-
V	No Brushing 187 PPM 96P 0.3% Sulframin AB No Food Soil	7	8.1 ±0.1	pH: 5.3±0.4 ppm Av. Cl. Init.: 183 Final: 176	pH: 5.1±0.3 ppm Av. Cl. Init.: 181 Final: 178	99.26	99.96	>99.99	>99.99	-
VI	15 Sec. Brushing 187 PPM 96P 0.3% Sulframin AB 0.1% Food Soil	6	8.1 ±0.2	pH: 5.2 ppm Av. Cl. Init.: 180 Final: 165	pH: 5.2 ppm Av. Cl. Init.: 181 Final: 181	99.95	-	-	99.98	>99.99
VII	15 Sec. Brushing 187 PPM 96P 0.3% Sulframin AB 0.1% Food Soil	23	7.8 ±0.2	pH: 5.1±0.1 ppm Av. Cl. Init.: - Final: 170	pH: 4.9 ppm Av. Cl. Init.: - Final: 179	99.97	-	>99.99	100.00	-

* Each value for 5 seconds is the mean of 4 or more experiments; those values for other exposure periods are means of 1 to 3 experiments.

** 0.3% in wash; .012% in each rinse.

reduction in count is largely of a physical nature and that while Sulframin AB possesses appreciable bacteriostatic activity, any germicidal activity is of a low order. After rinsing for 120 seconds each in rinses 1 and 2, there was a reduction of about 98% and similar results were obtained following the rinsing for 300 seconds in both rinses 1 and 2.

One procedure of chemical sanitizing employed by the Air Force has been to use G.I. soap as a wash and "Pittchlor" (calcium hypochlorite) for rinsing. To simulate this procedure, a solution of 0.3% G.I. soap was prepared for the wash and each of the two rinses was prepared with 50 ppm available chlorine of "Pittchlor." All solutions were held at about 7°C. Table XI, line II, indicates that 15 second brushing in the G.I. soap, and 5 second rinsing in each of the two rinses (50 ppm available chlorine) showed a reduction of 99.85%. Rinsing for 120 seconds in each rinse resulted in 99.94% reduction whereas rinsing for 300 seconds in each rinse resulted in a reduction of greater than 99.99%.

When the concentration of "Pittchlor" was increased to 200 ppm available chlorine in each rinse, holding the concentration of G.I. soap in the wash at 0.3% and holding the temperature of all solutions low (8°C.) there was greater than 99.99% reduction after only 5 second rinsing in each of the two rinses. As would be expected, when similar experiments were carried out at 25°C. likewise a 99.99% reduction was obtained after 5 second rinsing in each rinse.

The effect of brushing of utensils is illustrated in Table XI by comparing line III with line V which indicates greater than 99.99% reduction when brushing is employed as compared with 99.26% in the absence of brushing. Employing about 187 ppm available chlorine of Chloromelamine 96P in each rinse, with 0.3% Sulframin AB in the wash at 7°C (Table XI, line VI) a reduction of 99.95% of the test organisms was secured after 5 seconds in each rinse and a

TABLE XII

PERCENTAGE REDUCTION OF *Micrococcus pyogenes* var. *aureus* ON SPOONS AFTER TREATMENT

		Temp. °C.	pH Wash	Rinse No.1	Rinse No.2	Exposure (seconds) in Each Rinse*				
						5	15	60	120	300
I	15 Sec. Brushing Sulframin AB** No Germicide	6	8.0 ±0.3	pH: 7.9±0.2 ppm Av.Cl. Init.: - Final: -	pH: 7.9±0.2 ppm Av.Cl. Init.: - Final: -	98.30	-	-	97.88	99.10
II	15 Sec. Brushing 50 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	7	10.5 ±0.1	pH: 8.1 ppm Av.Cl. Init.: 38 Final: 25	pH: 7.9 ppm Av.Cl. Init.: 38 Final: 27	99.71	-	-	>99.99	99.93
III	15 Sec. Brushing 187 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	8	10.6 ±0.1	pH: 8.5±0.1 ppm Av.Cl. Init.: 164 Final: 147	pH: 8.3±0.1 ppm Av.Cl. Init.: 168 Final: 153	99.99	-	>99.99	>99.99	-
IV	15 Sec. Brushing 187 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	24	10.5 ±0.1	pH: 8.0±0.1 ppm Av.Cl. Init.: 166 Final: 121	pH: 8.0±0.1 ppm Av.Cl. Init.: 166 Final: 128	>99.99	-	>99.99	>99.99	-
V	No Brushing 187 PPM 96P 0.3% Sulframin AB No Food Soil	7	8.1 ±0.1	pH: 5.3±0.4 ppm Av.Cl. Init.: 183 Final: 176	pH: 5.1±0.3 ppm Av.Cl. Init.: 181 Final: 178	99.54	99.86	99.95	>99.99	-
VI	15 Sec. Brushing 187 PPM 96P 0.3% Sulframin AB 0.1% Food Soil	6	8.1 ±0.2	pH: 5.2 ppm Av.Cl. Init.: 180 Final: 165	pH: 5.2 ppm Av.Cl. Init.: 181 Final: 181	99.89	-	-	>99.99	99.98
VII	15 Sec. Brushing 187 PPM 96P 0.3% Sulframin AB 0.1% Food Soil	23	7.8 ±0.2	pH: 5.1±0.1 ppm Av.Cl. Init.: - Final: 170	pH: 4.9 ppm Av.Cl. Init.: - Final: 179	>99.99	-	>99.99	>99.99	-

* Each value for 5 seconds is the mean of 4 or more experiments; those values for other exposure periods are means of 1 to 3 experiments

** 0.3% in wash; .012% in each rinse.

TABLE XIII

PERCENTAGE REDUCTION OF *Micrococcus pyogenes* var. *aureus* ON FORKS AFTER TREATMENT

		Temp. °C.	pH Wash	Rinse No.1	Rinse No.2	Exposure (Seconds) In Each Rinse*					
						5	15	30	60	120	300
I	15 Sec. Brushing Sulframin AB** No Germicide	6	8.0 ±0.3	pH: 7.9±0.2 ppm Av.Cl. Init.: - Final: -	pH: 7.9±0.2 ppm Av.Cl. Init.: - Final: -	96.99	-	-	-	97.94	99.88
II	15 Sec. Brushing 50 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	7	10.5 ±0.1	pH: 8.1 ppm Av.Cl. Init.: 38 Final: 25	pH: 7.9 ppm Av.Cl. Init.: 38 Final: 27	99.83	-	-	-	>99.99	>99.99
III	15 Sec. Brushing 187 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	8	10.6 ±0.1	pH: 8.5±0.1 ppm Av.Cl. Init.: 164 Final: 147	pH: 8.3±0.1 ppm Av.Cl. Init.: 168 Final: 153	>99.99	-	-	100.00	100.00	-
IV	15 Sec. Brushing 187 PPM Pittchlor 0.3% G.I. Soap 0.1% Food Soil	24	10.5 ±0.1	pH: 8.0±0.1 ppm Av.Cl. Init.: 166 Final: 121	pH: 8.0±0.1 ppm Av.Cl. Init.: 166 Final: 128	>99.99	-	-	100.00	>99.99	-
V	No Brushing 187 PPM 96P 0.3% Sulframin AB No Food Soil	7	8.1 ±0.1	pH: 5.3±0.4 ppm Av.Cl. Init.: 183 Final: 176	pH: 5.1±0.3 ppm Av.Cl. Init.: 181 Final: 178	99.81	99.91	99.95	>99.99	>99.99	-
VI	15 Sec. Brushing 187 PPM 96P 0.3% Sulframin AB 0.1% Food Soil	6	8.1 ±0.2	pH: 5.2 ppm Av.Cl. Init.: 180 Final: 165	pH: 5.2 ppm Av.Cl. Init.: 181 Final: 181	99.96	-	-	-	>99.99	>99.99
VII	15 Sec. Brushing 187 PPM 96P 0.3% Sulframin AB 0.1% Food Soil	23	7.8 ±0.2	pH: 5.1±0.1 ppm Av.Cl. Init.: - Final: 170	pH: 4.9 ppm Av.Cl. Init.: - Final: 179	>99.99	-	-	100.00	>99.99	-

* Each value for 5 seconds is the mean of 4 or more experiments; those values for other exposure periods are means of 1 to 3 experiments.

** 0.3% in wash; .012% in each rinse.

reduction of more than 99.99% was obtained after 300 seconds in each rinse. When a similar experiment was conducted at a higher temperature, 23°C., (line VII) a reduction of 99.97% was obtained after 5 seconds in each rinse and a reduction of 99.99% after 60 seconds in each rinse.

Somewhat similar results were found for the disinfection of spoons and forks as noted in Tables XII and XIII.

Conclusions

A G.I. soap wash followed by two rinses (7°C) each containing "Pittchlor" at 50 ppm available chlorine produced a bacterial reduction of 98.85% on meat cans, 99.71% on spoons and 99.83% on forks. At a "Pittchlor" concentration of 187 ppm available chlorine (8°C.) the reduction was 99.99% for all three utensils. Increasing the temperature from 8°C. to 24°C. at 187 ppm available chlorine likewise resulted in 99.99% reduction for each type of utensil.

The use of Sulframin AB in the wash and Chloromelamine 96P at 187 ppm available chlorine in the rinse (6°C.) showed bacterial reductions of 99.95% for meat cans, 99.89% for spoons and 99.96% for forks. The same detergent and germicide used at 23°C. showed a reduction of 99.97% for meat cans and 99.99% for spoons and forks. These results indicate that Sulframin AB and Chloromelamine 96P at comparable concentrations and temperatures are slightly less efficient than G.I. soap and "Pittchlor". Chloromelamine 96P in two 5-second rinses produced a 99.99% bacterial reduction under some conditions, but did not do so under all conditions of test.

Part E

Disinfection of Raw Vegetables

Introduction

The purpose of this study was to determine the efficacy of Chloromelamine Formula 96P as a raw vegetable disinfectant for military use in areas where available fresh fruits and vegetables may have been exposed to contamination by night soil, sewage effluents or other sources of pathogens affecting humans.

A limited review of the literature revealed several papers concerned with vegetable disinfection. The work of Rudolfs and his associates (1), (2), Hogg (3), Mills (4), Mellick (5), Dunlop (6) and Slocum and Boyle (7) was helpful in the selection of procedures. From the information available it appeared that the blending of whole vegetables for examination was not feasible because:

(1) The process of blending incorporated into the mixture a large volume of air in the form of bubbles making it difficult to determine exact volumes.

(2) With tomatoes, seeds, parts of the skin, and the pulp frequently made it impossible to pipette samples and thus affected the reliability of the determinations.

(3) The use of a vegetable blend necessitated the use of a buffer to control pH effects of the highly-acid tomato and with blends of other vegetables introduced the possible antibacterial effects of vegetable juices.

(4) The literature indicated that the inner tissue of raw wholesome and unbruised vegetables was free of bacteria. The primary information sought, concerned the efficacy with which solutions of Chloromelamine 96P killed microorganisms of possible intestinal origin occurring on the surface of raw vegetables.

Procedure

1. General.

The plan of study was based on directions for the disinfection of fresh fruits and vegetables outlined in Military Specification MIL-D-11309 (QMC), 6 August 1951 (used in lieu of Jan-C-1054, 28 March 1949), pages 17 and 18.

"For fresh fruits and vegetables:

a. Remove bruised and torn outer leaves and damaged fruits or vegetables.

b. Do not cut or peel fruits or vegetables before disinfecting.

c. Wash thoroughly in a solution made by dissolving the contents of this package in 20 gallons of warm water, (100°F.).

d. After washing, completely immerse the fruits or vegetables for 30 minutes in a separate freshly prepared solution made by dissolving 1 package of disinfectant, chlorine, food service, in 20 gallons of warm water (100°F.). Stir occasionally to obtain thorough wetting of vegetables and fruit surfaces.

e. After 30 minutes of immersion, remove the fruits and vegetables from the disinfectant solution and rinse thoroughly in potable water."

In the laboratory studies an attempt was made to simulate the disinfection of fresh vegetables as carried out in the field. Our geographical location and the season precluded the possibility of obtaining supplies of fresh vegetables grown under conditions where they were likely to have become contaminated with feces, sewage effluents or other possible sources of pathogens. For this reason all the studies were conducted on fresh vegetables artificially contaminated with a culture of Escherichia coli (EHC-198, ATCC #11229) used as a simulant for the enteric pathogens.

Except for the studies on lettuce, the blending of vegetable samples for examination was discarded in favor of a rinsing technique. An estimation was made of the presence and density of coliform organisms by the "Most Probable Number" technique (confirmed test) as outlined in APHA "Standard Methods for the Examination of Water and Sewage" Ninth Edition, 1946. A final test for residual coliform organisms on the vegetable surface after disinfection and rinsing was made by incubating the whole vegetable in brilliant green bile broth containing a fermentation tube.

A preliminary evaluation of Chloromelamine Formula 96P as a vegetable disinfectant was made, using experimentally inoculated tomatoes, carrots, celery and lettuce. For purposes of comparison, the efficacy of a hypochlorite as a germicidal rinse was made at 25°C. The hypochlorite compound used was trade named "Pittchlor" and supplied by the Surgeon's Office, Air Material Command, Wright-Patterson Field, Dayton, Ohio. It was labeled as a "Germicide and Disinfectant" with the following formula:

Active Ingredient	
Calcium Hypochlorite	70%
Inert Ingredients	30%

High quality tomatoes, carrots, celery and lettuce were purchased from a Cincinnati wholesale produce company. Because surface area was not considered, vegetables of uniform size or weight by market standards were chosen for the study. They were as follows:

Tomatoes - No. 4 size. Four tomatoes per tube with an average weight of 100 grams each.

Carrots - Approximately 6 inches in length.

Celery - No. 10 Stalks (10 dozen per crate)

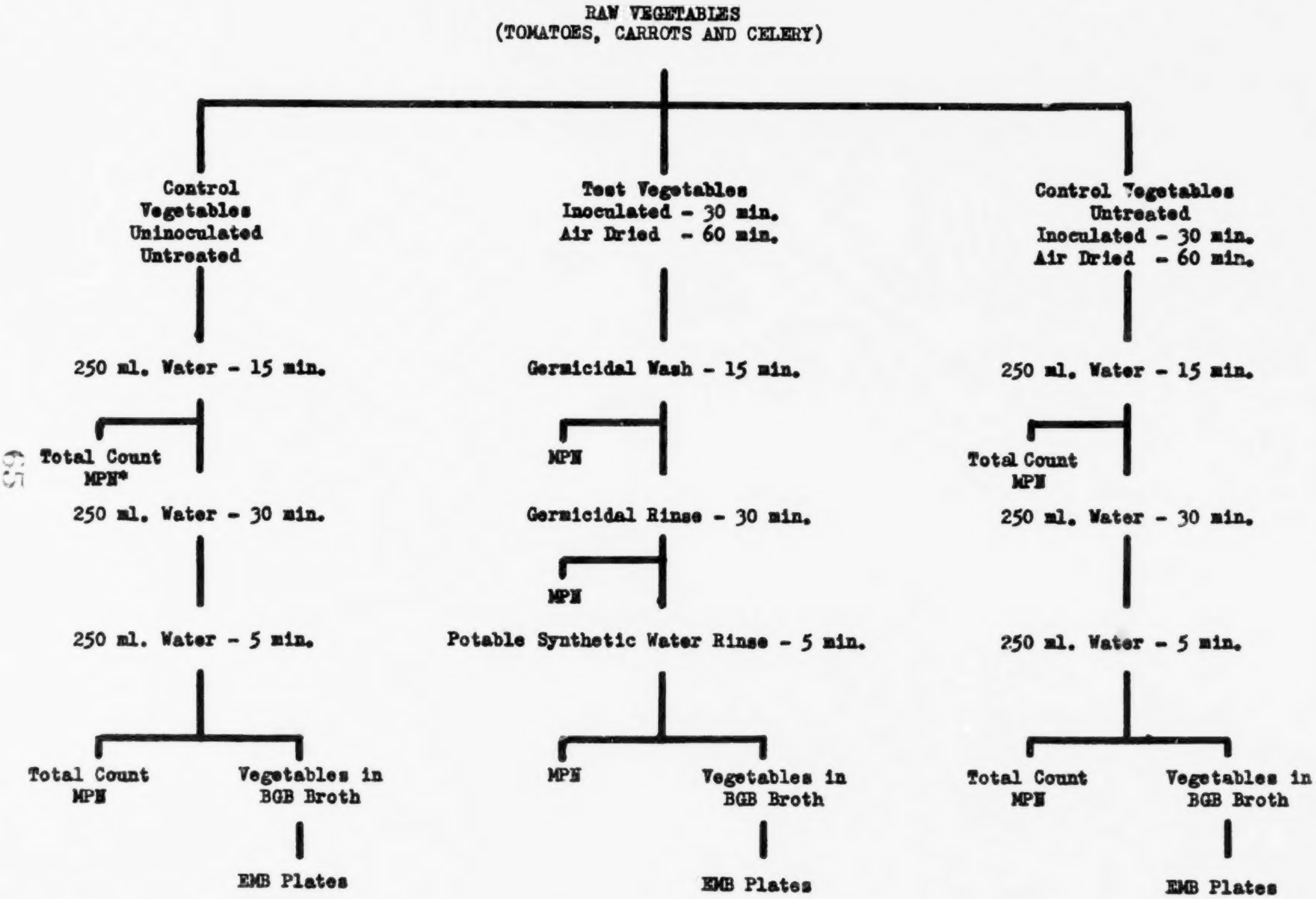
Lettuce - No. 5 Heads (5 dozen per crate)

In accordance with "Recommended Military Characteristics" for a "Germicidal Rinse Compound For Field Use Of The Armed Forces", reproduced on pages 1 and 2, the vegetable disinfection studies were conducted at 5°C. and at 25°C. rather than at 50°C. upon request of the Air Material Command. The water used throughout this study was a synthetic hard water also specified in "Military Characteristics". The 0.2 per cent peptone was omitted from the water formulation since the vegetables undergoing disinfection constituted a high organic loading.

Chloromelamine Formula 96P was studied as a vegetable disinfectant at the concentration specified by Q.M.C. Military Specifications (3.35 oz. net in 20 gallons of water). This resulted in solutions containing approximately 200 ppm available chlorine. The hypochlorite "Pittchlor" was tested at a concentration of approximately 50 ppm available chlorine. Initial and final pH determinations and chlorine titrations were made of the germicidal wash and rinse solutions by the prescribed methods. The pH determinations were made with a glass electrode and chlorine titrations with 0.10 N sodium thiosulfate solution.

Figure 6 is a flow sheet showing the procedure followed in the disinfection, sampling and testing of inoculated tomatoes, carrots, and celery. Figure 7 shows the revised general procedure followed in the disinfection of lettuce. The types of controls and their treatment are also shown.

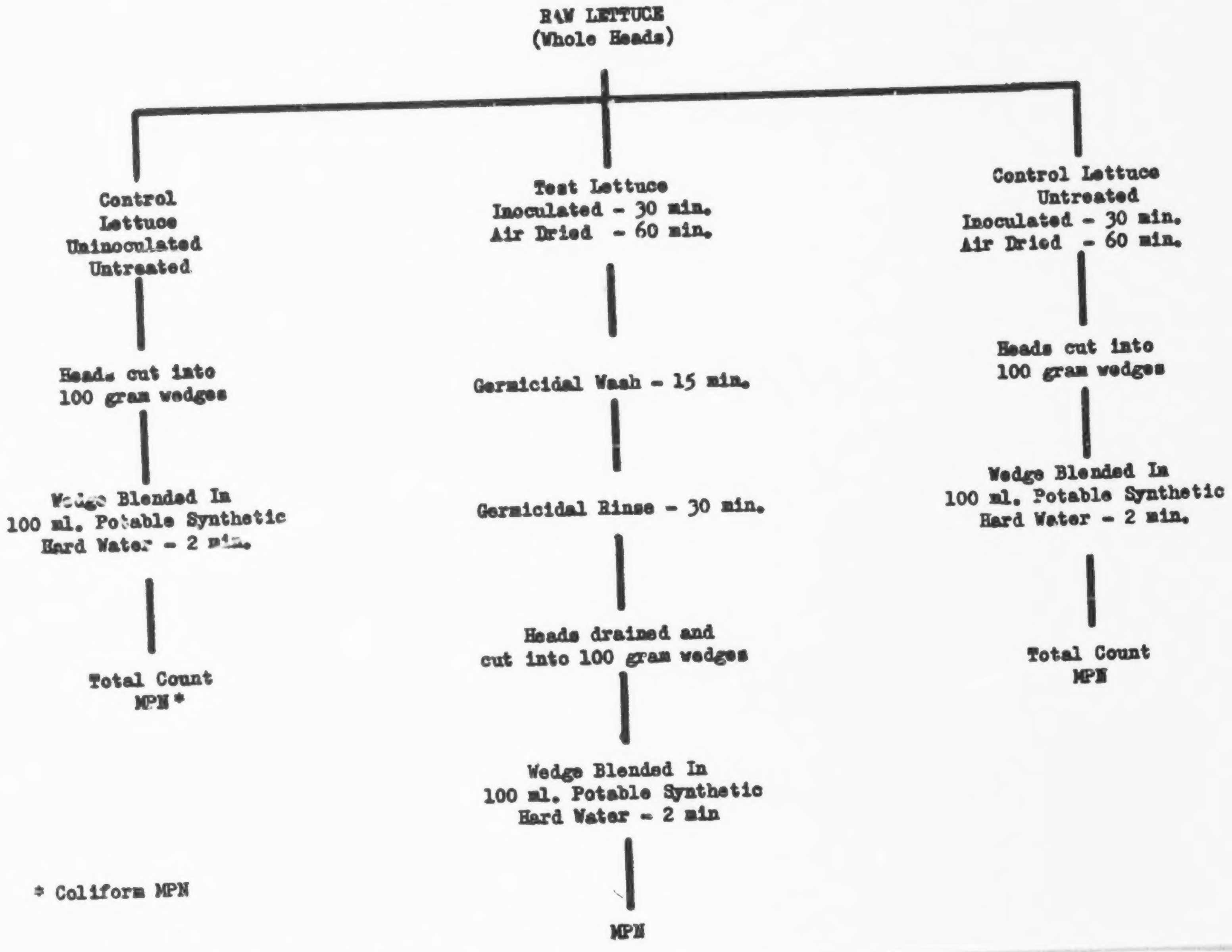
FLOW SHEET OF PROCEDURES USED
IN TESTING THE EFFICIENCY OF CHLOROMELAMINE FORMULA 96P
AS A RAW VEGETABLE DISINFECTANT


RAW VEGETABLES
(TOMATOES, CARROTS AND CELERY)
Control Vegetables Uninoculated Untreated
Test Vegetables Inoculated - 30 min. Air Dried - 60 min.
Control Vegetables Untreated Inoculated - 30 min. Air Dried - 60 min.
250 ml. Water - 15 min.
Germicidal Wash - 15 min.
250 ml. Water - 15 min.
Total Count MPN*
MPN
Total Count MPN
250 ml. Water - 30 min.
Germicidal Rinse - 30 min.
250 ml. Water - 30 min.
MPN
250 ml. Water - 5 min.
Potable Synthetic Water Rinse - 5 min.
250 ml. Water - 5 min.
Total Count MPN
Vegetables in BGB Broth
MPN
Vegetables in BGB Broth
Total Count MPN
Vegetables in BGB Broth
EMB Plates
EMB Plates
EMB Plates

* Coliform MPN

FIGURE 7

FLOW SHEET OF PROCEDURES USED
IN TESTING THE EFFICIENCY OF CHLOROMELAMINE FORMULA 96P
AS A RAW VEGETABLE DISINFECTANT


RAW LETTUCE
(Whole Heads)
Control
Lettuce
Uninoculated
Untreated
Heads cut into
100 gram wedges
Wedge Blended In
100 ml. Potable Synthetic
Hard Water - 2 min.
Total Count
MPN *
Test Lettuce
Inoculated - 30 min.
Air Dried - 60 min.
Germicidal Wash - 15 min.
Germicidal Rinse - 30 min.
Heads drained and
cut into 100 gram wedges
Wedge Blended In
100 ml. Potable Synthetic
Hard Water - 2 min
MPN
Control Lettuce
Untreated
Inoculated - 30 min.
Air Dried - 60 min.
Heads cut into
100 gram wedges
Wedge Blended In
100 ml. Potable Synthetic
Hard Water - 2 min.
Total Count
MPN

* Coliform MPN

2. Base-line studies on Cincinnati market vegetables.

To establish base-line data on the bacterial count and the presence of coliform organisms on the surface of fresh tomatoes, carrots, celery and lettuce, several lots of each vegetable were purchased on different days. Individual samples of tomatoes, carrots and celery were rinsed for five minutes in suitable receptacles (beakers or graduates) containing 250 ml. of sterile water. Total counts of the organisms washed off the surface of vegetables were made by plating appropriate amounts of rinse water in tryptone glucose extract agar. The coliform MPN was determined by "Standard Methods", confirmed test. Tests for residual coliform organisms which adhered to the vegetable surface, were made by incubating the whole vegetable in brilliant green bile broth containing a fermentation tube. Growth and gas formation in 48 hours was followed by making streak plates on Levine's Eosine Methylene Blue agar, these incubated and read for the presence of typical colonies of *E. coli* (Plates II and III).

With market head lettuce, 100 gram wedges were sampled, placed in sterile Waring blender jars containing 100 ml. of water and blended for 2 minutes. Total counts and coliform MPN determinations were made of the lettuce-water blend.

In all determinations, the estimated plate counts per ml. and the MPN per 100 ml. of rinse water were calculated to express the results as total bacteria and the coliform MPN per 250 ml. of rinse water or the total count and coliform MPN per vegetable sample. For lettuce the results were converted to total count and coliform MPN per 100 gram wedge.

3. Inoculation of Test Vegetables.

The culture suspension for the artificial contamination of the vegetables was prepared by washing with distilled water the growth from 18 to 24 hour nutrient agar slant cultures grown at 35°C. Sufficient E. coli cell suspension concentrate was added to four liters of sterile synthetic hard water to result in a final count of 100,000,000 organisms per ml.

The unwashed test vegetables were prepared for inoculation as follows:

Tomatoes - Unbruised tomatoes weighing approximately 100 grams.

Carrots - Carrots approximately six inches in length with foliage removed.

Celery - Base of stalk removed and lower six inch segments of celery strips used.

Lettuce - Whole heads with bruised and discolored outer leaves removed.

The vegetables were submerged for 30 minutes in the E. coli culture suspension in a sterile glass battery jar or a four gallon zinc dipped can. At 5 minute intervals the vegetables and culture suspension were agitated. The test vegetables were then removed individually, placed on a sterile wire-mesh rack and air dried for 60 minutes. The method of experimental contamination of the test vegetables is illustrated in Plate I.

4. Disinfection of Inoculated Vegetables.

In accordance with Q.M.C. Military Specifications the contaminated vegetables were washed in a solution of Formula 96P, (Germicidal Wash Solution), then transferred to a second solution of Formula 96P, (Germicidal Rinse Solution), and completely immersed for 30 minutes. The disinfected vegetables were then transferred individually to receptacles containing 250 ml. of a sterile synthetic hard water with sufficient 0.10 N sodium thiosulfate to neutralize any residual chlorine ("Potable Water").

TEST METHOD

DISINFECTION OF VEGETABLES

CHLOROMELAMINE 96P

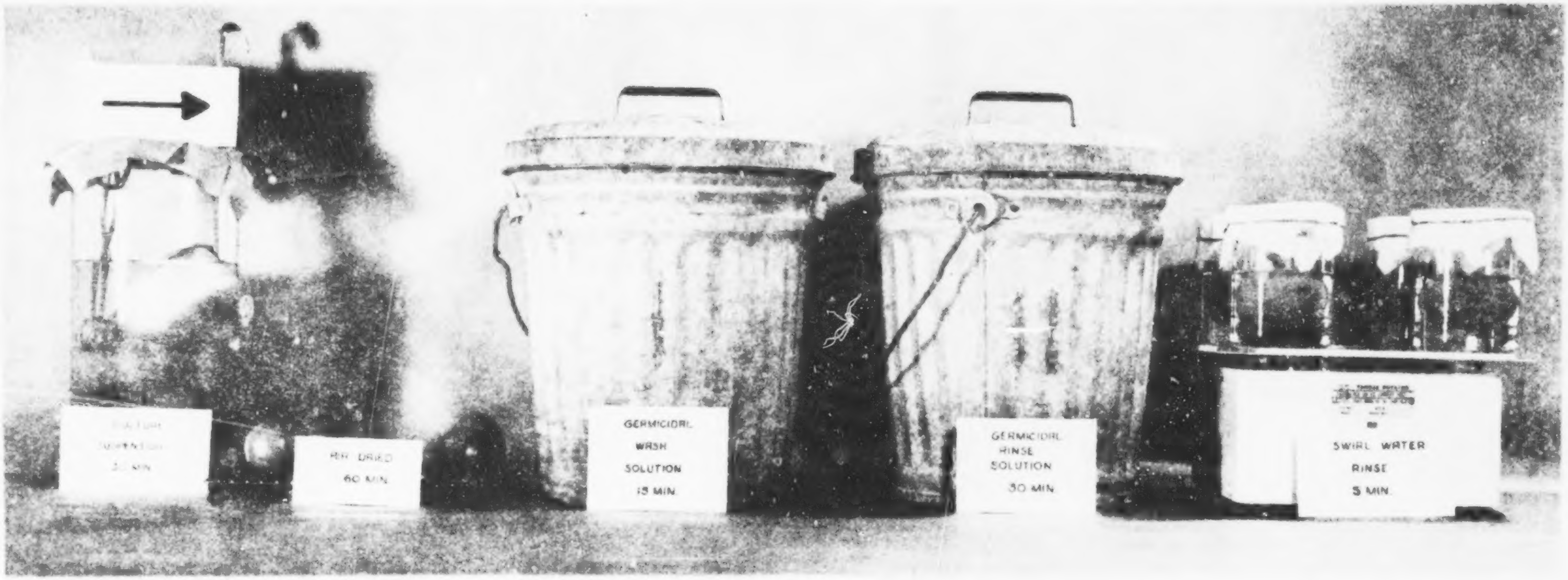

CULTURE
SUSPENSION
AIR DRIED
60 MIN
GERMICIDAL
WASH
SOLUTION
15 MIN
GERMICIDAL
RINSE
SOLUTION
30 MIN
SWIRL WATER
RINSE
5 MIN

PLATE I

TEST METHOD

DISINFECTION OF VEGETABLES

CHLOROMELAMINE 96P

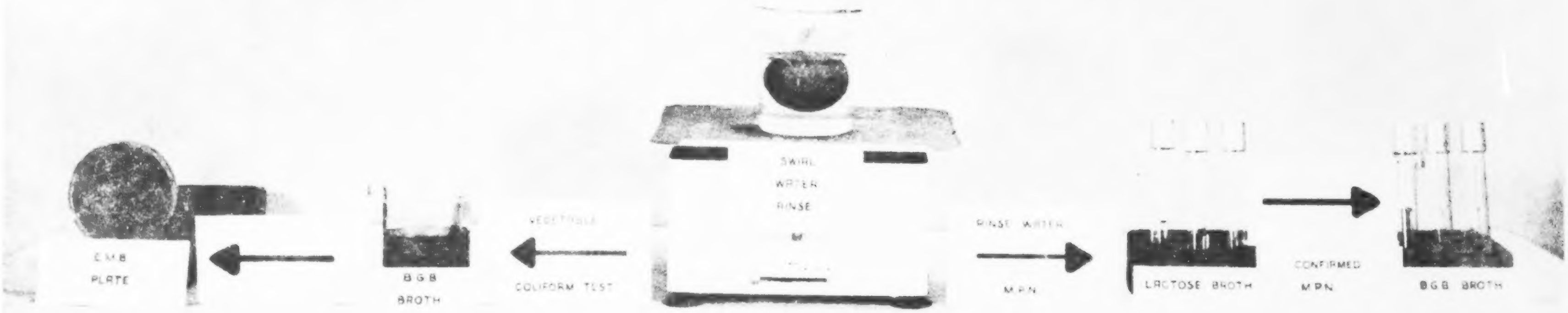

E.M.B
PLATE
B.G.B
BROTH
VEGETABLE
COLIFORM TEST
SWIRL
WATER
RINSE
RINSE WATER
M.P.N.
LACTOSE BROTH
CONFIRMED
M.P.N.
B.G.B. BROTH

PLATE II

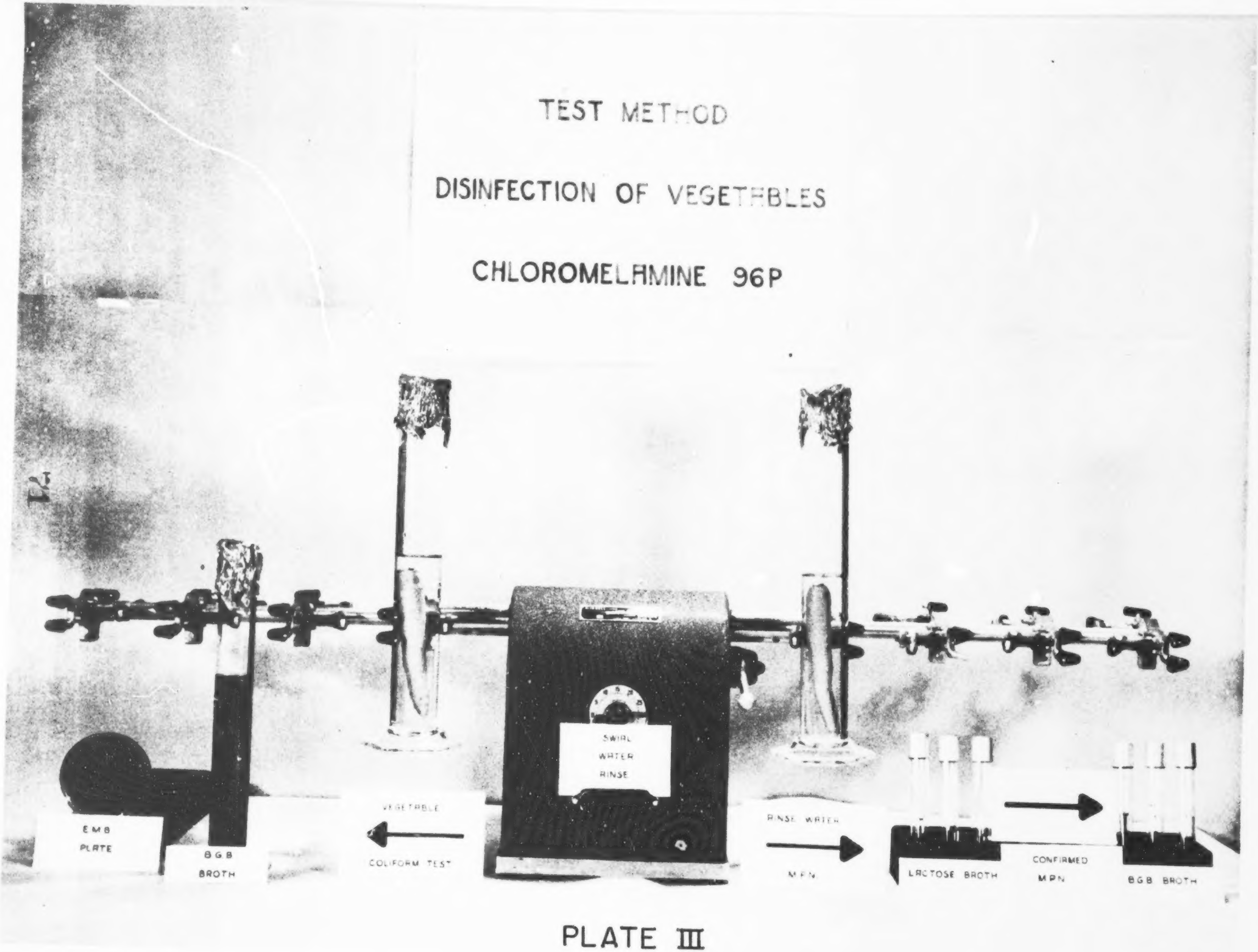

TEST METHOD
DISINFECTION OF VEGETABLES
CHLOROMELAMINE 96P
EMB PLATE
BGB BROTH
VEGETABLE
COLIFORM TEST
SWIRL WATER RINSE
RINSE WATER
MPN
LACTOSE BROTH
CONFIRMED MPN
BGB BROTH

PLATE III

After disinfection, tomatoes were rinsed in beakers containing 250 ml. of synthetic hard water for 5 minutes on Yankee Rotator* (Plate II). Disinfected carrots and celery strips were rinsed for 5 minutes in 500 ml. graduates containing a similar volume of rinse water on a "wrist action" shaker** as illustrated in Plate III.

It should be noted that the time fresh fruits and vegetables are held and the manner they are washed in the germicidal wash solution are not specified in Q.M.C. Military Specifications. Directions for rinsing the disinfected vegetables in "potable water" are likewise not specific. After exploratory studies the following disinfection holding periods were adopted:

Germicidal Wash Solution - 15 minutes

Germicidal Rinse Solution - 30 minutes (Q.M.C. Military Specifications)

Potable Water Rinse - 5 minutes

The only deviations from Q.M.C. Military Specifications were the 15 minute holding period in the germicidal wash solution (not specified by Q.M.C.) and the rinsing of individual test vegetable samples in 250 ml. of water with mechanical aids. Plate counts and coliform M.P.N. were determined of the water in which the disinfected vegetables were rinsed.

---

* Manufactured by the Clay Adams Co.,Inc., New York, N.Y.

** Manufactured by the Burrell Corp., Pittsburg, Pa.

With whole lettuce heads after 25 minutes' exposure in the germicidal rinse solution the heads of lettuce were removed, placed on a sterile wire-mesh rack to drain and 100 gram wedges sampled from the disinfected heads, weighed and immediately placed in sterile Waring blender jars containing 100 ml. of the rinse water and 0.10 N sodium thiosulfate. The draining, sampling and weighing were completed in 5 minutes to give a total elapsed exposure time to the disinfectant of 30 minutes as required by specifications. By exploratory studies it had been determined that 1.0 ml. of water per 1 gram of lettuce and a 2 minute blending period were necessary to give a mixture which could be pipetted. The final volume of the lettuce-water blend was 200 ml.

A plate count and coliform MPN was made of the lettuce-water blend. The results were converted to the total count and the coliform M.P.N. per 100 gram wedge of lettuce. The procedure is illustrated in Plate IV and in Figure 7.

5. Controls.

Two sets of controls were used in each disinfection test run. The controls consisted of (1) uninoculated market vegetables chosen at random and (2) samples of inoculated vegetables, which were not exposed to the germicide but held in 250 ml. portions of sterile water for the same time intervals as were used in the disinfection treatment of inoculated vegetables (15, 30 and 5 minutes). The flow sheets (Figures 6 and 7) show the procedure followed with the control vegetables. The rinsing of control vegetable samples in sterile waters for the same time intervals as the inoculated disinfected samples provided information on the total count, an estimate of the "Most Probable Number", of coliform

TEST METHOD

DISINFECTION OF VEGETABLES

CHLOROMELAMINE 96P

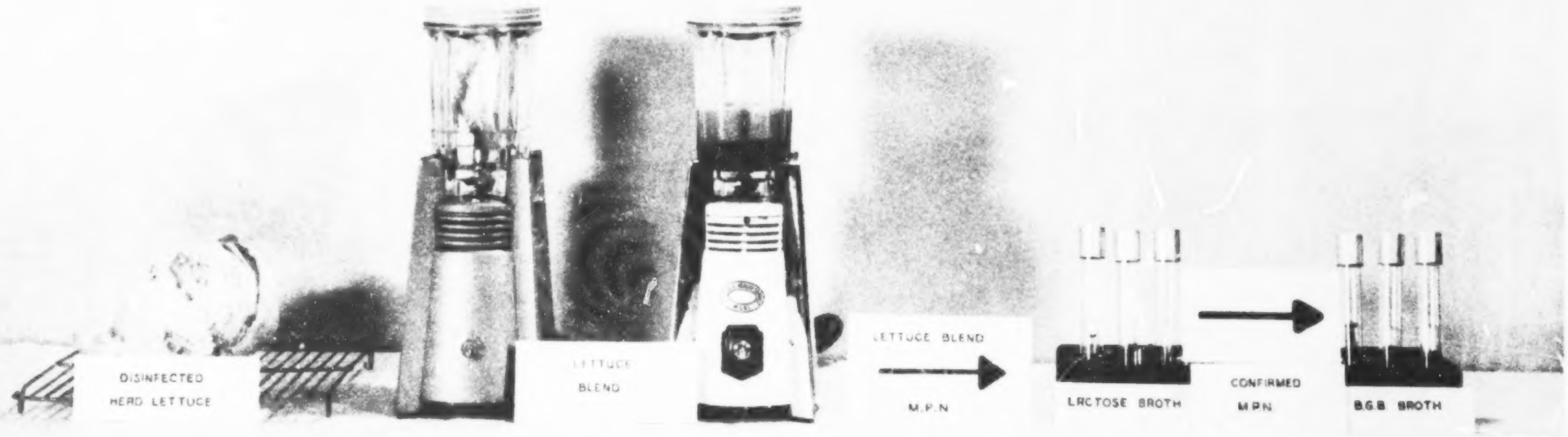

DISINFECTED
HEAD LETTUCE
LETTUCE
BLEND
LETTUCE BLEND
M.P.N
LACTOSE BROTH
CONFIRMED
M.P.N
B.G.B BROTH

PLATE IV

organisms on the surface of inoculated vegetables and the effect of rinsing on the removal of organisms from the surface of the vegetables.

All results were converted to the total count and coliform MPN per test vegetable.

Results

1. Fresh Market Vegetables.

A preliminary qualitative study of tomatoes and carrots showed that for 55 market tomatoes the median total count per sample was 500,000. Coliform organisms were present on all samples as evidenced by growth and gas formation in brilliant green bile broth. Fifty (50) carrot samples when tested in a similar manner showed a median total count of 3,000,000 and all samples were coliform positive.

The results of quantitative coliform tests on the raw market vegetables are shown in Table XIV. There was considerable variation in the total counts and coliform MPN. With market tomatoes there was a variation of three logs in total count and the coliform MPN ranged from <450 to >400,000. All 17 samples showed the presence of coliform organisms. Market lettuce samples (100 gram wedges) showed the lowest median coliform MPN (128) while market carrots showed the highest (55,000).

2. Disinfection of Tomatoes.

Total counts and coliform MPN were determined for twelve control inoculated tomatoes passed through sterile wash and rinse waters rather than through the germicidal wash, germicidal rinse and final potable water rinse. The median total count was 21,000,000 and the coliform MPN greater

TABLE XIV

TOTAL ORGANISMS AND COLIFORMS (MPN)
REMOVED FROM MARKET VEGETABLES BY RINSING

	Per Tomato		Per Carrot		Per Celery Strip		Per Lettuce Wedge	
Sample	Total Count	Est. MPN	Total Count	Est. MPN	Total Count	Est. MPN	Total Count	Est. MPN
1	$13x10^{6}$	6,000	$22x10^{5}$	42,500	$16x10^{5}$	1,875	$78x10^{5}$	144
2	$22x10^{7}$	> 400,000	$54x10^{5}$	70,000	$18x10^{5}$	500	$56x10^{6}$	7.4
3	$22x10^{5}$	195	$19x10^{6}$	> 400,000	$41x10^{5}$	1,950	$46x10^{6}$	7.4
4	$19x10^{6}$	13,500	$33x10^{5}$	230,000	$47x10^{5}$	500	$24x10^{6}$	96
5	$83x10^{4}$	1,950	$14x10^{6}$	> 400,000	$36x10^{5}$	8,250	$26x10^{6}$	3,200
6	$89x10^{4}$	< 450	$9x10^{4}$	27,500	$39x10^{6}$	19,750	$54x10^{6}$	9.0
7	$38x10^{4}$	< 450	$53x10^{4}$	7,950	$33x10^{6}$	27,500	$16x10^{7}$	7.4
8	$13x10^{6}$	450	$14x10^{5}$	3,250	$13x10^{6}$	42,500	$36x10^{6}$	9.0
9	$25x10^{5}$	5,800	$22x10^{5}$	87,500	$15x10^{6}$	450	$88x10^{6}$	128
10	$4x10^{6}$	< 450	$39x10^{5}$	37,500	$42x10^{5}$	3,250	$13x10^{7}$	82
11	$85x10^{5}$	8,300	$13x10^{5}$	16,000	$65x10^{5}$	12,250	$92x10^{6}$	> 3,200
12	$16x10^{6}$	32,500	$54x10^{5}$	8,250	$29x10^{6}$	70,000	$12x10^{7}$	> 3,200
13	$85x10^{6}$	> 40,000	$17x10^{6}$	5,750	$18x10^{6}$	230	$13x10^{7}$	> 3,200
14	$12x10^{6}$	23,000	$12x10^{7}$	400,000	$5x10^{6}$	50	$72x10^{6}$	3,200
15	$8x10^{6}$	23,000	$7x10^{7}$	> 400,000	$5x10^{6}$	195	$46x10^{6}$	700
16	$2x10^{6}$	8,750	$46x10^{6}$	> 400,000	$11x10^{6}$	1,975	$44x10^{6}$	20
17	$3x10^{6}$	2,375	$3x10^{7}$	55,000	$8x10^{6}$	825	$84x10^{6}$	1,080
18			$35x10^{6}$	55,000	$9x10^{5}$	50	$26x10^{5}$	3,200
19			$63x10^{7}$	> 400,000	$65x10^{4}$	< 45	$74x10^{2}$	64
20			$59x10^{5}$	45,000	$11x10^{6}$	425	$58x10^{4}$	> 3,200
21			$83x10^{5}$	42,500	$4x10^{6}$	4,250	$36x10^{6}$	4
22					$3x10^{6}$	113	$13x10^{6}$	128
23					$15x10^{5}$	2,750		
24					$19x10^{5}$	234		
Median	$4x10^{6}$	8,300	$59x10^{5}$	55,000	$4x10^{6}$	1,350	$46x10^{6}$	128

than 400,000 for eleven of the twelve samples. The median total count and coliform MPN per sample in the final "potable" water rinse was 920,000 and 40,000 respectively. The decrease in total count and MPN per sample after passage through sterile water solutions indicated the efficiency of the mechanical rinsing procedure.

To evaluate Chloromelamine Formula 96P as a vegetable disinfectant duplicate experiments were made at 5 and 25°C. using 10 inoculated tomatoes in each experiment. The results are shown in Table XV. The median coliform MPN per vegetable sample was 472 at 5°C. and 18.5 at 25°C. With a median coliform MPN of >400,000 for the inoculated untreated control samples, Formula 96P effected a reduction of the MPN of >99.9% at 5°C. and >99.99% at 25°C. In a single experiment using 10 inoculated tomatoes disinfected with the hypochlorite "Pittchlor" at 25°C. the median coliform MPN was 2,300 with an MPN reduction in excess of 99.4%.

3. Disinfection of Carrots.

In the experiments on the disinfection of carrots a total of 10 control inoculated samples were passed through "potable" waters instead of the disinfectant solutions. Removal of organisms from the inoculated carrots in the sterile wash water resulted in a median total count of 16,000,000 per vegetable sample and a coliform MPN greater than 400,000 for 9 control carrots. Examination of the final rinse waters gave a median total count of 2,100,000 organisms per sample. The median coliform MPN was indeterminate because 5 of the 10 control carrot samples showed a coliform MPN in excess of 400,000 and 4 with lesser MPN values.

TABLE XV

DISINFECTION OF EXPERIMENTALLY CONTAMINATED FRESH TOMATOES

Vegetable Sample	Temp. Of Test °C.	After Disinfection		Germicidal Wash Solution	Germicidal Rinse Solution
		Coliform MPN per Tomato	E. coli on Tomato Surface		
		CHLOROMELAMINE FORMULA 96P			
1	5	2,300	+	pH: 3.92 ppm Av.Cl. Initial:226 Final: 196	pH: 3.92 ppm Av.Cl. Initial:195 Final: 214
2		550	+		
3		4,000	+		
4		425	+		
5		425	+		
6		4,000	+		
7		20	+		
8		275	+		
9		400	+		
10		135	+		
11		350	+	pH: 3.87 ppm Av.Cl. Initial:192 Final: 199	pH: 4.07 ppm Av.Cl. Initial:194 Final: 192
12	6	230	+		
13		60	+		
14		1,350	+		
15		135	-		
16		2,300	-		
17		> 4,000	+		
18		520	+		
19		4,000	+		
20		> 4,000	+		
1	25	< 1.58	+	pH: 4.7 ppm Av.Cl. Initial:190 Final: 142	pH: 4.63 ppm Av.Cl. Initial:209 Final: 152
2		< 1.58	+		
3		10.5	+		
4		< 1.58	-		
5		< 1.58	+		
6		875	+		
7		45	+		
8		< 1.58	+		
9		17.25	-		
10		1.68	+		
11	25	> 4,000	+	pH: 3.89 ppm Av.Cl. Initial:199 Final: 166	pH: 3.92 ppm Av.Cl. Initial:193 Final: 174
12		11.5	+		
13		> 4,000	-		
14		< 1.58	+		
15		60	+		
16		19.75	-		
17		275	+		
18		> 4,000	-		
19		27.5	+		
20		875	+		
		PITTCHLOR			
1	25	875	+	pH: 8.25 ppm Av.Cl. Initial:58 Final: 54	pH: 7.91 ppm Av.Cl. Initial:54 Final: 37
2		400	+		
3		4,000	+		
4		400	+		
5		2,300	+		
6		87.5	+		
7		4,000	+		
8		2,300	+		
9		> 4,000	+		
10		2,300	+		

In the disinfection of experimentally contaminated carrots a single experiment was conducted at 5°C. and duplicate experiments at 25°C. Ten carrot samples were used in each experiment. The results are shown in Table XVI. Coliform MPN tests on the final rinse water at 5°C. resulted in a median MPN of 4000 and at 25°C. in a median MPN of 488. At 5°C. Formula 96P disinfection reduced the median MPN from <400,000 to 4000 or a reduction of approximately 99.0% while at 25°C. the reduction of coliform MPN was >99.9%.

The hypochlorite did not appear to be as effective a germicidal agent as Formula 96P since 9 of the 10 samples gave coliform MPN values of <4000.

4. Disinfection of Celery Strips.

Eight control inoculated celery strips were passed through sterile wash and rinse waters instead of the germicidal solutions. The median total count per celery strip was 24,000,000 and 2,600,000 respectively. The coliform MPN values were indeterminate. After passage through the sterile wash water the coliform MPN for four celery strips was >4,000,000 and for the remaining four strips >400,000. After passage through the final sterile rinse water the coliform MPN per celery strip was still >400,000.

In the disinfection of inoculated celery strips groups of 10 strips each were exposed to Chloromelamine Formula 96P solutions. Duplicate experiments were made at 25°C. and a single experiment at 5°C. With the hypochlorite a single experiment at 25°C. was conducted. Thus a total of 40 inoculated celery strips were disinfected, 30 with Formula 96P and 10 with the hypochlorite.

TABLE XVI

DISINFECTION OF EXPERIMENTALLY CONTAMINATED FRESH CARROTS

Vegetable Sample	Temp. Of Test °C.	After Disinfection		Germicidal Wash Solution	Germicidal Rinse Solution
		Coliform MPN per Carrot	E. coli on Carrot Surface		
		CHLOROMELAMINE FORMULA 96P			
1	5	4,000	+	pH: 3.84	pH: 4.01
2		4,000	+	ppm Av.Cl.	ppm Av.Cl.
3		4,000	+	Initial:189	Initial:157
4		4,000	+	Final: 191	Final: 200
5		4,000	+		
6		4,000	+		
7		4,000	+		
8		4,000	+		
9		4,000	+		
10		4,000	+		
1	25	875	+	pH: 3.81	pH: 3.81
2		425	+	ppm Av.Cl.	ppm Av.Cl.
3		175	+	Initial:231	Initial:225
4		2,300	+	Final: 199	Final: 194
5		425	+		
6		425	+		
7		1,350	+		
8		425	+		
9		2,300	+		
10		4,000	+		
1	25	40,000	+	pH: 4.05	pH: 4.13
2		120	+	ppm Av.Cl.	ppm Av.Cl.
3		550	+	Initial:232	Initial:237
4		1,075	+	Final: 200	Final: 189
5		115	+		
6		2,300	+		
7		< 5	+		
8		300	+		
9		60	+		
10		600	+		
		PITTCHLOR			
1	25	> 4,000	+	pH: 8.07	pH: 7.88
2		> 4,000	+	ppm Av.Cl.	ppm Av.Cl.
3		> 4,000	+	Initial:52	Initial:61
4		> 4,000	+	Final: 36	Final: 50
5		> 4,000	+		
6		> 4,000	+		
7		875	+		
8		> 4,000	+		
9		> 4,000	+		
10		> 4,000	+		

In 35 of the 40 treated celery strips the coliform MPN was indeterminate, being greater than 4000. In only 3 of 30 inoculated celery strips disinfected with Formula 96P was the coliform MPN reduced to less than the median coliform MPN (1,350) of the market celery strips.

5. Disinfection of Lettuce.

In the control series in which heads of lettuce were inoculated with E. coli, drained and 100 gram wedges sampled and blended the median total count per 100 gram wedge was 1,700,000,000 and the coliform MPN >32,000,000.

In the disinfection of inoculated heads of lettuce with Formula 96P duplicate experiments were conducted at 5 and 25°C. With the hypochlorite germicide a single experiment was made at 25°C. Six heads of lettuce were used in each experiment. Indeterminate MPN values were obtained which exceeded a coliform MPN of 3,200,000 despite the use of greatly increased dilutions of the lettuce blend in conducting the test. In none of the sample wedges from inoculated heads disinfected with either germicide was the coliform MPN reduced to less than that of the market lettuce samples.

Table XVII summarizes the range and MPN values of market control vegetables and vegetables inoculated with E. coli and disinfected with Formula 96P at 5 and 25°C. Head lettuce purchased on the open market when sampled and tested by the method described was low in coliform organisms. This may be explained by the fact that the outer leaves serve as a protective covering and prevent contamination of the inner structure of the head. The normal removal of the bruised and discolored outer leaves further reduced the number

TABLE XVII

COMPARATIVE RANGE AND MEDIAN COLIFORM MPN VALUES OF CONTROL AND DISINFECTED VEGETABLES(1)

Type of Vegetable	Controls Market Raw Vegetables No. of Samples	Range	Median	Experimentally Contaminated and Disinfected Vegetables Chloromelamine 96P - 5°C. No. of Samples	Range	Median	Chloromelamine 96P - 25°C. No. of Samples	Range	Median
Tomatoes	17	< 450- > 400,000	8,300	20	20- > 4,000	472	20	< 2- > 4,000	18.5
Carrots	21	3,250- > 400,000	55,000	10	4,000 (Constant)	4,000	20	< 5- 40,000	488
Celery	24	< 45- 70,000	1,350	10	1,350- > 4,000	*	20	875- > 4,000	*
Lettuce	22	4- > 3,200	128	12	*	*	12	*	*

* Indeterminate - In the majority of samples the MPN was >400,000.

(1) Results expressed as organisms per vegetable for tomatoes and carrots. Results for lettuce expressed as organisms per 100 gram wedge, for celery as organisms per 6-inch strip.

of organisms. The root vegetable, carrot, had the highest concentration of surface coliform organisms. In the disinfection of tomatoes and carrots with Formula 96P the germicide was more effective at 25 than at 5°C. When tomatoes and carrots were inoculated with *E. coli* and exposed to the germicidal solutions for a total of 45 minutes (germicidal wash solution 15 minutes, and germicidal rinse solution 30 minutes) the coliform MPN was reduced below the coliform MPN found on the surface of the control market vegetables. Due to indeterminate MPN endpoints obtained in the tests with celery and lettuce the range and MPN median were not reportable.

Conclusions

(1). A rinsing and test procedure was devised to determine the presence and density of coliform organisms on the surface of vegetables.

(2). There was considerable variation in the total counts and coliform MPN of market vegetable samples. All market vegetable samples tested showed the presence of coliform organisms by the confirmed test. In increasing order the median coliform "Most Probable Number" was 128 for 22 samples of lettuce wedges, 1,350 for 24 samples of celery strips, 8,300 for 17 tomato samples and 55,000 for 21 carrot samples.

(3). When Formula 96P (200 ppm available chlorine) was used as a germicidal rinse it reduced the median coliform MPN from >400,000 for the inoculated untreated control tomato samples to a median of 472 at 5°C. and 18.5 at 25°C. or a reduction of >99.9% and >99.99% respectively. In the disinfection of carrots at 5 and 25°C. Formula 96P effected a reduction of the median coliform MPN of 99.0% and >99.9%. In the disinfection of celery and lettuce the numbers of inoculated organisms remaining after disinfection were much greater than those found on control market samples.

(4). Increasing the temperature at which the germicidal rinse was used, from 5 to 25°C. increased the germicidal activity markedly.

(5). The disinfection of inoculated tomatoes and carrots at 5 and 25°C. resulted in a reduction of the median coliform MPN values below those for the market control samples.

(6). At 25°C. the hypochlorite germicidal rinse was not as effective as Chloromelamine Formula 96P.

Bibliography

(1) Rudolfs, Willem, Falk, Lloyd L., and Ragotzkie, Robert A.: Contamination of vegetables grown in polluted soil. I - Bacterial Contamination. Sew. and Indust. Wastes 23: 253-268 (1951). II - Field and Laboratory studies on *Endamoeba* cysts. Sew. and Indust. Wastes 23: 478-485 (1951). III - Field studies on *Ascaris* eggs. Sew. and Indust. Wastes 23:656-660 (1951). IV - Bacterial decontamination. Sew. and Indust. Wastes 23: 739-751 (1951). V - Helminthic decontamination. Sew. and Indust. Wastes 23: 853-860 (1951). VI - Application of results. Sew. and Indust. Wastes 23: 992-1000 (1951).

(2) Rudolfs, W. et. al.: Literature review on the occurrence and survival of enteric, pathogenic, and relative organisms in soil, water, sewage, and sludges, and on vegetation. I - Bacterial and virus diseases. Sew. and Indust. Wastes 22: 1261-1281 (1950).

(3) Hogg, E.S.: The washing of watercress grown in sewage effluent. J. & Proc. Ind. Sew. Purif. 239 (1944).

(4) Mills, R., Bartlett, C.L., and Kessel, J.F.: The penetration of fruit and vegetables by bacteria and other particulate matter, and the resistance of bacteria, protozoan cysts and helminth ova to common disinfection methods. Am. J. Hyg. 5: 559 (1925).

(5) Mellick, C.O.: The possibility of typhoid infections through vegetables. J. Inf. Dis. 21: 28 (1917).

(6) Dunlop, Stewart G., Univ. of Colo., Dept. of Medicine.: The incidence and significance of intestinal pathogens in sewage-contaminated irrigation water and on food products irrigated with this polluted water. I - Progress report, March 1, 1950. II - Supplemental progress report, May 1, 1950. III - Progress report, February 1, 1951 to October 15, 1951.

(7) Slocum, G., and Boyles, W.A.: Incidence of coliform bacteria on fresh vegetables. Food Res. 6:377-385 (1941).

SECTION III

Chemical and Physical Properties
of
Chlorome1amine Formulations

Part A

Introduction

It is the purpose of this section to present some studies on the physical and chemical characteristics of trichloromelamine mixtures and the shelf stability of a mixture packaged in glass bottles, paper cardboard boxes and plastic bags, when stored at temperatures varying from 160°F. to minus 65°F. and at relative humidities up to 100 per cent.

When one mole of melamine is chlorinated with three moles of chlorine, trichloromelamine ($C_3N_3(NHCl)_3$) is formed. This compound has a theoretical available chlorine content of 0.9274 grams per gram of trichloromelamine. Chlorination to 1.29 grams of available chlorine per gram of compound results in a more water insoluble and less stable compound whereas chlorination to 0.92 to 0.96 grams of available chlorine per gram of compound results in a more soluble and stable product. The latter product gradually releases free chlorine when dissolved in water. Free chlorine is known to have high bactericidal properties so chloromelamine has been proposed as a general disinfectant, particularly useful where hypochlorites or other oxidants are rapidly dissipated by impurities in the solution to be disinfected (1).

In order to enhance the solubility and obtain a favorable pH for disinfection, the trichloromelamine is usually mixed with a surfactant and buffer salt. The component parts of the proposed disinfectant contain chloromelamine, Sulframin AB -85, NaH_2PO_4 and citric acid. The shelf stability tests were made with Formula 96P specifications of the Office of the Army Quartermaster General, dated March 2, 1951. This formulation specifies by weight 22 per cent chloromelamine, 11.5 to 12.5 surface active material, 51 to 55 per cent anhydrous citric acid and 10.5 to 15.5 per cent anhydrous monosodium acid phosphate.

---

(1) National Research Council Subcommittee Report on Water Supply Bulletin, pp. 169-190, February 12, 1951.

Part B

Basic Studies

Part B

Basic Studies

1. Methods

In the studies, the component parts of the Quartermaster formula were separately dissolved in distilled water and mixed with chloromelamine in varying proportion to determine

(a) the free chlorine by the Wallace and Tiernan Amperometric test,(2) and

(b) the total available chlorine by a starch-iodide titration method similar to the American Public Health Association Standard Methods technique for residual chlorine.

In the shelf stability tests the free and available chlorine tests were similarly made by the amperometric and starch-iodide methods, respectively.

Procedure

In the preliminary tests on the stability of chloromelamine formulations prepared in the laboratory, the proposed concentration of 1,000 ppm was made by weighing out 1.00 gram of powder and transferring it to a volumetric flask. Distilled water was added to exactly one liter. The flask was rapidly inverted ten times before immediately removing a 25 ml. portion with a volumetric pipette for available chlorine test and a 100 ml. aliquot for a free chlorine test. The stock suspension was set aside for an interval of 30 minutes or more before the test was repeated. On repeating the test the suspension was mixed by inversion of the flask and pipetting aliquots just as in the initial test. This same procedure wasused for tests repeated after one hour, two hours and 20 hours. In addition, an aliquot of the stock suspension was removed one to two hours after preparation and heated to 30°F. until the suspended matter was largely dissolved before sub-samples were tested for free and available chlorine. The technique was further improved after 100 days storage by titrating a weighed sample of approximately 0.2500 g. in 250 ml. water warmed during the titration to assist in the solution. A low iodine titer was maintained during the solution of chloromelamine.

---

(2) Amperometric Methods in the Control of Water Chlorination, H.C. Marks and G.L. Bannister, Anal. Chem. 19, 200 (1947)

Since it was difficult to obtain representative samples for total available chlorine, due to rapidly settling suspensions, the technique was altered prior to testing Chloromelamine 96P. The improved technique which was adopted for use was as follows:

(1) One gram of chloromelamine was weighed, transferred to one liter volumetric flask and diluted with distilled water to one liter. The mixture was mixed by inversion ten times.

(2) A volume of 135 to 140 ml. was filtered through the asbestos mat in a Gooch crucible with the aid of suction. The suspension was mixed by inversion during the filtration period of two to three minutes.

(3) For dissolved available chlorine, 25 ml. of filtrate was immediately transferred by means of a pipette to a 300 ml. Erlenmeyer flask which contained 60 ml. of distilled water, 10 ml. of 1:1 glacial acetic acid and 10 ml. of potassium iodide reagent (0.76 g. KI). The flask is shaken briskly and titrated with standard (approximately) 0.01 N) sodium thiosulfate to the starch-iodide endpoint.

(4) For free chlorine - 100 ml. of residual filtrate, 100 ml. distilled water, and 5 ml. of 0.1 molar phosphate buffer at pH 7.0 were added to a Wallace and Tiernan amperometer vessel and immediately titrated with standard phenylarsene oxide (1 ml. equivalent to 0.1 mg. free Cl_2) to the minimum conductance endpoint.

(5) The above tests were repeated on the initial prepared suspension after standing for one, two and 24 hours.

(6) Total available chlorine - one gram of mixture was added to one liter of water and heated to 80°F. until most of the powder was dissolved. The sample was then cooled and a 25 ml. aliquot of the turbid mixture was taken for an iodometric titration as previously described.

2. The Composition of Chloromelamine Formulations

Three formulations of chloromelamine designated as 55-5, MQ (Modified Quartermaster) and 96P were used. The composition by weight in these formulations is as follows:

	FORMULATION		
	55-5	MQ	96P
	Per Cent	Per Cent	Per Cent
Chloromelamine	56	22	22
Sulframin, A.B.-85	4.6	11.5	12
NaH_2PO_4	39.4	9.3	13
Citric Acid	-	37.4	53
NaCl	-	19.8	-
Total	100.0	100.0	100

Formulation 55-5 was used for a solubility and stability test. Formulation MQ was used for solubility, stability of solutions and the effect of chloromelamine solution on citric acid and peptone prior to the receipt of Formula 96P several months after the studies had begun. It will be noted that 20 per cent of weight of MQ is sodium chloride whereas the 96P formula is richer in sodium acid phosphate and citric acid by an equivalent amount. Formulation 96P was used for shelf stability and solution stability tests.

Solubility and Stability of Chloromelamine 55-5

Weighed amounts of Chloromelamine 55-5 were suspended in one liter of distilled water. The total available chlorine was tested after one to two minutes and again after 24 hours. The solutions were only slightly cloudy after preparation. The results obtained are shown in Table XVIII. The solution containing 0.4 grams per liter was retested after ten minutes when 202 ppm available chlorine was found. The results show that this formulation was quite soluble in concentrations up to 300 ppm available chlorine or 150 per cent of the proposed concentration for normal use. Within the limits of the test procedure, the available chlorine found corresponds to the trichloromelamine in the formula. The available chlorine in dilutions varying from 25 to 300 ppm appeared to be stable during a 24 hour contact period.

3. Comparative Stability of Solutions of Chloromelamine and Hypochlorous Acid

Distilled water solutions of chlorine as hypochlorous acid and chloromelamine MQ were allowed to stand at room temperature for two hours and during this interval available chlorine was periodically determined on sub-samples. Similarly, citric acid or peptone was added separately to distilled water solutions of the two chlorine-bearing reagents and the available chlorine was tested periodically.

TABLE XVIII

THE SOLUBILITY OF CHLOROMELAMINE 55-5

Chloromelamine 55-5	Available Chlorine ppm		
g. per liter	Theoretical*	After 1-2 Minutes	After 24 Hours
0.05	27.4	28	27
0.1	51.8	51	50
0.2	106	102	100
0.4	211	176	208
0.6	316	293	298

(*) Based on trichloromelamine

TABLE XIX

COMPARATIVE STABILITY OF SOLUTIONS OF CHLOROMELAMINE (MQ) AND HYPOCHLOROUS ACID (Cl_2) IN WATER AND SOLUTIONS OF PEPTONE AND CITRIC ACID

Reaction Time Hours	Available Chlorine in ppm					
	Distilled Water		Citric Acid(1)		Peptone(2)	
	Cl_2	MQ	Cl_2	MQ	Cl_2	MQ
0	198	189(3)	200	189(3)	200	189(3)
0.1	-	-	204	169	114	166
0.25	197	168	165	163	101	153
0.50	197	169	128	151	97	130
1.0	196	167	82	132	90	148
2.0	194	152	35	144	85	156
4.0	-	-	-	-	73	-

(1) 374 ppm in the Cl_2 test and a total of 589 ppm in the MQ test.

(2) 1,000 ppm

(3) Theoretical concentration of 204 ppm available chlorine as trichloromelamine.

TABLE XX

STABILITY OF CHLOROMELAMINE 55-5 AND 96P IN PEPTONE SOLUTIONS

Reaction Time	55-5 and Peptone Solution(1)		MQ and Sulframin(2)	96P and water (3)		96P and Peptone (4)	
Hours	Available Cl_2 ppm	Free Cl_2 ppm	Available Cl_2 ppm	Available Cl_2 ppm	Free Cl_2 ppm	Available Cl_2 ppm	Free Cl_2 ppm
0	191	18.8	200	189	8.2	182	3.4
0.1	187	0.0	217	-	-	165	0.6
0.25	180	0.0	217	168	5.8	160	0.2
0.5	182	Tr.	214	169	5.4	163	0.0
1.0	177	0.2	214	167	4.4	-	-
2.0	172	0.1	210	152	5.7	161	0.8
20.0	122	0.6	-	-	4.2	94	0.7

(1) 400 ppm Peptone

(2) 119 ppm sulframin A.B.-85

(3) At pH 3.0

(4) 1,000 ppm Peptone

(2) When sulframin in a concentration 115 ppm more than the original formula is present in the chloromelamine, the mixture yields a higher available chlorine content than the theoretical 204 ppm calculated for trichloromelamine.

(3) The free chlorine liberated from one gram of chloromelamine 96P per liter of distilled water varied from 4.2 to 8.2 ppm, whereas the available chlorine in solution diminished to 152 ppm in the two hour test period.

(4) The presence of 1,000 ppm of peptone destroyed all but a few tenths of a part per million of the free chlorine in Chloromelamine 96P solution. After two hours the amperometric titration indicated about 0.7 ppm of free chlorine.

The foregoing preliminary studies are presented as an indication of the physical and chemical characteristics of several chloromelamine preparations.

4. The Shelf Stability of Chloromelamine 96P When Packaged in Glass Bottles, Cardboard Boxes and Plastic Bags.

Samples of Chloromelamine 96P were packaged in 100 gram quantities in:

(a) Four-ounce glass bottles with bakelite screw caps.

(b) Six-ounce cardboard boxes.

(c) Four-ounce polyphene plastic bags.

Four sample lots of each type were stored in incubators or refrigerators at the Wright-Patterson Air Base beginning September 11, 1951.

The storage conditions were:

(1) 75± 5°F. and normal humidity (these samples were stored at the Environmental Health Center, Faller Building, Cincinnati, Ohio).

(2) 35± 5°F. and normal humidity.

(3) 65°F. below zero and normal humidity.

(4) 160°F. and 6 per cent relative humidity.

(5) 160°F. and 90 to 100 per cent relative humidity.

Periodically, sets of the three packages were removed for test purposes.

Storage at 75°F. or Lower

Sets of samples were tested after 0, 12.3, 97, 158 and 192 days storage at room temperature, after 15.9, 64, and 78, 113, 127 and 175 days storage at 32°F., and after 15.9, 78, 113 and 175 days storage at 65°F. below zero. After 100 days storage the mixture packaged in boxes was lumpy and had a brownish color. The contents of some bottles was also lumpy. The contents of plastic bags stored for 175 to 192 days appeared the same as when packaged.

The effect of storage on the liberation of free chlorine upon solution is presented in Table XXI. In each case, one gram of Chloromelamine 96P was added to one liter of distilled water. The pH was found to be 3.0. The available chlorine reported in column 5 represents a sample of the solution after heating to 80°F. for two hours. Since the solutions were still turbid, the sample may not be representative due to settleable insoluble oxidant.

The free chlorine present in solution after two minutes and one, two and 20 hours at room temperature is presented in columns 6 to 9, respectively. The free chlorine in the solution heated to 80°F. is shown in column 10.

The results show that storage for less than 200 days at temperatures of 68°F. or lower the free chlorine of all samples varied from 0.1 to 8.2 ppm. Usually more than one and less than 5 ppm of free chlorine were found in unheated samples more or less independently of the solution time. Heating to 80°F. usually liberated a significant amount of additional free chlorine.

TABLE XXI

EFFECT OF STORAGE ON THE LIBERATION OF FREE CHLORINE FROM CHLOROMELAMINE

Storage Conditions				Available Cl_2* ppm (5)	Free Chlorine in Solution After				
Temp. °F. (1)	R.H. % (2)	Time Days (3)	Container (4)		2 min. ppm (6)	1 Hr. ppm (7)	2 Hrs. ppm (8)	20 Hrs. ppm (9)	2 Hr. Heated ppm (10)
68	N	0.5	Bot.	187(1)	8.2	4.4(2)	5.7	4.2	-
		0	Bot.	-	4.8	2.2	4.2	4.2	-
			Box	-	1.9	3.0	3.8	3.7	-
			Bag	-	2.3	3.5	4.0	3.6	-
		12.3	Bot.	177	0.6	1.1	1.6	3.4	6.1
			Box	183	1.4	2.4	2.7	1.9	5.4
			Bag	169	2.9	2.9	3.2	3.7	5.3
		97	Bot.	171	0.6	1.5	1.7	1.6	2.5
			Box	169	1.1	1.3	2.0	1.8	2.6
			Bag	161	1.1	1.4	1.8	1.8	2.6
		158	Bot.	186	1.2	1.4	1.3	1.7	
			Box	153	1.5	1.6	1.3	1.6	
			Bag	170	1.2	1.2	1.8	2.9	
		192	Bot.	177	0.6	1.0	1.8	1.8	
			Box	156	1.4	1.9	2.1	2.1	
			Bag	182	2.4	2.5	2.6	3.2	
32	N	15.9	Bot.	187	5.8	2.7	2.5	3.0	7.2
			Box	175	3.1	3.0	3.4	2.3	3.3
			Bag	183	3.6	2.3	4.0	3.2	7.2
		64	Bot.	171	1.6	2.6	2.6	0.0	4.5
		78	Bot.	183	0.7	1.3	1.6	1.9	1.7
			Box	178	1.7	1.7	3.2	2.8	3.3
			Bag	177	1.8	2.6	2.9	3.0	2.7
		113	Bot.	183	0.8	1.3	2.9	4.9	
			Box	176	1.4	1.8	2.9	2.5	
			Bag	182	1.5	2.8	3.6	5.3	
		127	Bot.	180	0.6	1.1	1.4	2.9	
			Box	175	1.1	1.6	1.8	3.6	
			Bag	186	1.7	2.8	3.6	3.9	
		175	Bot.	188	0.1	1.8	2.6	2.8	
			Box	163	2.0	2.9	2.7	3.0	
			Bag	182	2.1	2.3	2.1	2.7	

(continued)

TABLE XXI (continued)

EFFECT OF STORAGE ON THE LIBERATION OF FREE CHLORINE FROM CHLOROMELAMINE

Storage Conditions				Available Cl_2* ppm (5)	Free Chlorine in Solution After				
Temp. °F. (1)	R.H. % (2)	Time Days (3)	Container (4)		2 min. ppm (6)	1 Hr. ppm (7)	2 Hrs. ppm (8)	20 Hrs. ppm (9)	2 Hr. Heated ppm (10)
-65		15.9	Bot.	167	1.9	3.3	3.4	3.6	4.1
			Box	177	4.1	3.0	4.4	4.4	4.7
			Bag	188	3.4	4.9	5.5	4.6	6.8
		78	Bot.	173	1.5	0.9	1.2	-	1.6
			Box	172	1.6	1.3	1.5	-	1.2
			Bag	168	3.1	1.7	2.8	-	1.7
		113	Bot.	179	0.4	0.6	0.7	1.7	
			Box	174	0.2	0.5	0.6	3.6	
			Bag	182	0.3	0.7	0.7	3.6	
		175	Bot.	182	0	2.3	2.6	4.9	
			Box	168	1.7	2.0	2.9	4.7	
			Bag	191	2.3	3.3	3.9	4.5	
160	6	14.2	Bot.	128	0.4	0.4	0.6	1.0	1.6
			Box	90	0.4	0.3	0.1	0.5	0.3
			Bag	152	0.3	0.5	0.7	1.3	1.6
		32	Bot.	78	0.0	0.0	0.04	0.0	0.28
			Box	63	0.0	0.0	0.0	0.08	0.08
			Bag	113	0.0	0.08	0.09	0.41	0.85
		77	Bot.	57	0.13	0.12	0.14	0.19	0.06
			Box	78	0.09	0.12	0.12	0.33	0.26
			Bag	112	0.12	0.15	0.18	-	0.56
		112	Bot.	71	0.2	0.2	0.1	0.1	
			Box	14	0.00	0.06	0.08	0.04	
			Bag	81	0.26	0.16	0.18	0.18	
		174	Bot.	57	0.8	1.2	2.3	0.8	
			Box	18	0.00	0.30	0.46	0.15	
			Bag	47	0.00	0.00	0.76	0.46	
160	95	0.1	Box	0(3)	0.0	-	-	-	-
		1.2	Bot.	8.4-10.9	0.0	0.0	0.0	0.0	0.0
		26.6	Bot.	0(4)	0.0	0.0	0.0	0.0	0.0
		26.6(5)	Bot.	4.9	0.03	0.01	0.0	-	0.0

(continued

TABLE XXI (continued)

EFFECT OF STORAGE ON THE LIBERATION OF FREE CHLORINE FROM CHLOROMELAMINE

(*) Found after dissolving in solution heated to 80°F.

(1) Average of four tests.

(2) Residual free Cl_2 of 0.24 and 0.0 ppm observed after 15 and 30 minutes, respectively.

(3) Residual available Cl_2 of 10.8 ppm found immediately on solution was dissipated by heating at 80°F.

(4) Residual available Cl_2 of 21.7 ppm found immediately on solution was dissipated by heating at 80°F.

(5) Retested December 27, 1951 after room temperature storage for 20 days.

The loss of available chlorine is more clearly shown in Table XXII where the percentage loss has been calculated from the original available chlorine of 187 mg. per gram of mixture before storage and the available chlorine in unfiltered samples heated to 80°F. Losses up to ten per cent were not considered significant. The available chlorine in the filtered solution after contact with one gram of chloromelamine mixture per liter of distilled water varied with the time of contact and frequency of mixing. After two minutes of mixing one gram of mixture in a liter of water at 68°F. sub-samples were filtered and the available chlorine in the filtrate determined. The amount found was expressed as a percentage of the original available chlorine of 187 milligrams per gram of mixture (value for bottle at 68°F. in Table XXI) are shown in Table XXIII. The test was repeated following solution of the Chloromelamine 96P after 0.5, 1, 2, and 20 hours at laboratory temperature. The results showed a progressive increase in dissolved available chlorine. These results show that 30 to 50 per cent of the available chlorine was dissolved in a few minutes as compared to 70 to 85 per cent after a solution period of 20 hours providing the chloromelamine had not been damaged by heat. At 160°F. the lesser percentage of dissolved available chlorine was due to losses during storage and possibly a decreased solubility of the product.

Storage at 160°F. and 90-100 per cent Relative Humidity

Two hours (0.1 days) after storage at 160°F. and 90-100 per cent relative humidity, enough chlorine gas had been evolved from the samples to make the odor in the incubator obnoxious and to interfere with other tests in progress. The very badly damaged cardboard boxes were removed from storage and inspected.

TABLE XXII

AVAILABLE CHLORINE LOSSES BY STORAGE

Storage Conditions			Percentage Loss of Original Chlorine*		
Temp. °F.	Relative Humidity Per cent	Time Days	In Bottle	In Box	In Bag
68	N(1)	12.3	5	2	10
		97	9	10	16
		158	1	18	9
		192	5	17	3
32	N(1)	15.9	0	6	2
		64	9	-	-
		78	2	5	6
		113	2	6	3
		127	4	6	1
		175	0	13	3
-65	-	15.9	11	8	0
		78.0	8	8	10
		113	4	7	3
		175	3	10	0
160	6	14.2	32	52	19
		32	58	66	40
		77	70	58	40
		112	62	92	57
		174	70	90	75
160	95(2)	0.1		93(4)	-
		1.2	94-96(3)	-	-(5)
		26.6	100	-	-
		26.6	96.5(3)	-	-

(continued)

TABLE XXII (continued)

AVAILABLE CHLORINE LOSSES BY STORAGE

(*) Original available chlorine = 187 mg. per g. of mixture at zero storage time.

(1) "Normal" laboratory relative humidity during September and October 1951.

(2) 90 to 100 per cent relative humidity.

(3) Based on residual found two minutes after preparing a suspension.

(4) Cardboard boxes badly damaged, mixture brownish black.

(5) Plastic bags almost completely destroyed by action of mixture.

TABLE XXIII

AVAILABLE CHLORINE IN SOLUTION

Storage Conditions			Percentage of Original Available Chlorine					
			Solution in 2 Minutes			Solution in 20 Hours		
Temp. °F.	Relative Humidity Per cent	Time Days	Bottle	Box	Bag	Bottle	Box	Bag
68	N	0	30	45	51	74	72	80
		12.3	35	41	44	74	81	81
		97	40	48	55	77	82	79
		158	41	47	48	70	68	75
		192	27	48	54	86	74	81
32	N	15.9	44	55	65	74	76	82
		64	30	-	-	72	-	-
		78	35	50	59	74	77	76
		113	34	50	49	70	77	87
		127	40	51	64	73	82	82
		175	35	52	47	62	70	73
-65	-	15.9	49	69	72	83	83	85
		78	38	53	70	75	78	76
		113	47	56	55	70	75	75
		175	41	34	56	72	71	[illegible]
160	6	14.2	42	32	43	67	45	70
		32	25	21	36	29	27	45
		77	21	18	25	27	27	38
		112	27	6	27	24	7	26
		174	17	6	8	22	8	10
160	95	0.1	7.4	-	-	-	-	-
		1.2	1.6	-	-	1.8	-	-
			4.8	-	-	6.3	-	-
		26.6	11.6	-	-	1.9	-	-
		26.6	3.5	-	-	2.2	-	-

The boxes were charred as by a fire and the chloromelamine mixture had a gingerbread appearance. Photographs were taken of these samples (Plate V). The entire contents (100 grams) of one box was added to two liters of water and passed through a colloid mill. Two aliquot samples were tested and the results indicated that the entire box contained only 28 to 40 milligrams of available chlorine. Another box which was darker in color was similarly tested and found to contain no available chlorine.

Samples of the plastic bags and two bottles were removed after storage for 28 hours (1.2 days). All but one of the plastic bags was badly damaged and the powder was dark brown and caked. The cap on one bottle was broken. Another bottle had a loose cap. Two bottles were received after a storage period of 26.6 and another after storage for 32.6 days. Data on all samples stored at 160°F. and 95 per cent relative humidity for two hours or more (See Tables XXI, XXII and XXIII) had lost not only the free chlorine but nearly all of the available chlorine. The condition of all of these samples indicated that the material would not stand storage at 160°F. at high relative humidity and further storage under these conditions was discontinued.

Storage at 160°F. and Six per cent Relative Humidity

A set of samples was tested after 14.2 days storage. The plastic bag was brittle and broken. The cardboard box was bleached. The bottle was not damaged. Additional samples were tested after 32, 77, 112 and 174 days storage. Samples stored at 160°F. and six per cent relative humidity for 14 days or more deteriorated in their ability to release free chlorine. Data on these samples are given in Tables XXI and XXII. Losses of 20 to 50 per cent of the available

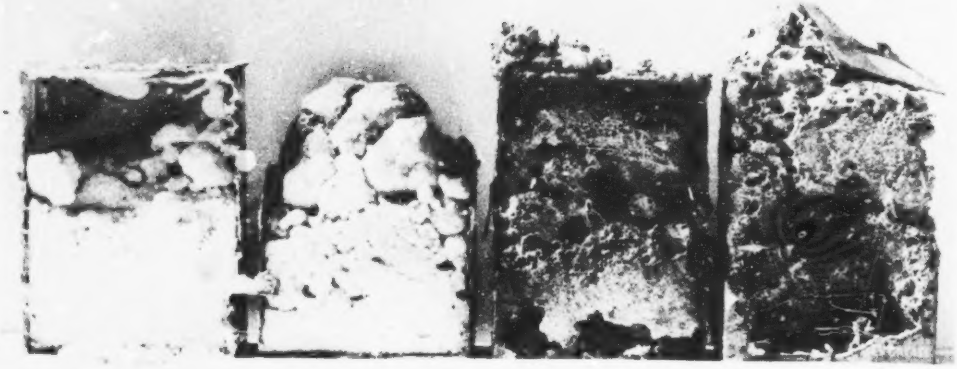


PLATE V

Chloromelamine disinfectant 96P packaged in cardboard boxes after exposure for two hours at 160°F. and 90 to 100 per cent relative humidity.

chlorine were found after 14 days, and losses of 40 to 92 per cent were found after prolonged storage. Although the chloromelamine does not keep well at this temperature and humidity, the plastic bag and glass bottle preserved the chloromelamine better than the cardboard box.

5. Summary and Discussion

The stability of solutions of chloromelamine and the shelf stability of Chloromelamine 96P, packaged in glass bottles, cardboard boxes and plastic bags for periods up to six months, was studied. The results indicate good shelf stability at room temperature to 65°F. below zero when packaged in bottles or plastic bags. Chloromelamine 96P deteriorated when stored over 150 days in cardboard boxes. Decomposition of chloromelamine was hastened at higher temperatures (160°F.) and with increased relative humidity. For example, in glass bottles at 160°F. and 6 per cent relative humidity, 32 per cent of the original chlorine was lost after 14 days. In contrast at a relative humidity of 95 per cent, a loss of 94 per cent of the original chlorine was observed after only 1.2 days. It appears, therefore, that the humidity is an important factor in the shelf life at temperatures above normal for this area.

The tests indicated that the cardboard container was the least suitable storage container. None of the packages were suitable for storage at 160°F. for more than a few months. The plastic container gave the longest, the bottle gave a lesser and the box gave the poorest shelf life for storage of the compound at temperatures of 68°F. or less.

Only a fractional part per million of free chlorine remained in solution when 1,000 ppm of peptone is present in water treated at room temperature with 1,000 ppm Chloromelamine 96P. This is true in the interval from a few minutes to an hour or more after adding the chloromelamine. This indicates that the effectiveness of the compound as a sanitizer, such as dishwashing rinses, would decrease with an increase of food particles containing protein and amino acid groups. Although citric acid has a high hypochlorous acid demand in the range 35 to 200 ppm available chlorine, the citric acid is not attacked nearly as rapidly by chloromelamine. Contrary to some literature(1), the wetting agent, sulframin at a concentration of 230 ppm does not appear to reduce the chlorine content of a chloromelamine solution containing 200 ppm available chlorine.

The study completed thus far indicates the need for additional work along the following lines:

(1) A continuation of the shelf stability tests at temperatures of 75°F. or less.

(2) Studies on the available chlorine of samples dried at 103°C. The stability of the mixture seems to deteriorate when the sample shows a loss of weight in excess of five per cent on drying. If the moisture content of the mixture is critical for stability, the incorporation of a desiccant in the mixture may improve the stability.

(3) Studies of the free and available chlorine and pH when water, sewage, sludge and process wastes are chlorinated with chloromelamine.

(4) Studies on the shelf life and applicability of a two-cell package whereby the chloromelamine and surfactant are kept apart from the buffer salt so that the stability may be preserved when stored at temperatures of 160°F. and 96 per cent relative humidity.

(5) Studies at temperatures below 160°F. to determine the maximum temperature for a reasonably long period of storage.

---

(1) National Research Council Subcommittee Report on Water Supply Bulletin, pp 169-190, February 12, 1951.